(51) International Patent Classification 5 : A61K 9/50, 49/00, 49/02, B01J 13/02	A1	(11) International Publication Number: WO 94/02106
		(43) International Publication Date: 3 February 1994 (03.02.94)

(21) International Application Number: PCT/US93/06675

(22) International Filing Date: 16 July 1993 (16.07.93)

(30) Priority data:
07/916,348   17 July 1992 (17.07.92)   US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: THERAPEUTIC AND DIAGNOSTIC USE OF MODIFIED POLYMERIC MICROCAPSULES
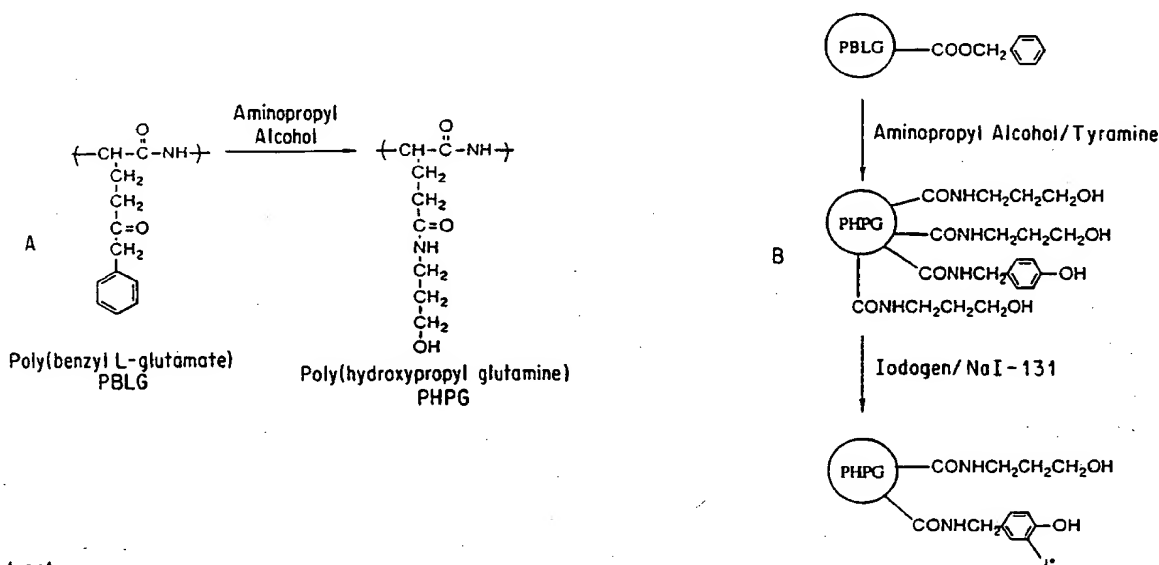
(57) Abstract

    The present invention relates to a highly efficient method of preparing modified microcapsules exhibiting selective targeting. These microcapsules are suitable for encapsulation or surface attachment of therapeutic and diagnostic agents. In one aspect of the invention, surface charge of the polymeric material is altered by conjugation of an amino acid ester to the polymer, providing improved targeting of encapsulated agents to specific tissue cells. Examples include encapsulation of hydrophilic radiodiagnostic agents in 1 μm capsules to provide improved opacification and encapsulation of cytotoxic agents in 100 μm capsules for chemoembolization procedures. The microcapsules are suitable for attachment of a wide range of targeting agents, including antibodies, steroids and drugs, which may be attached to the microcapsule polymer before or after formation of suitably sized microcapsules. The invention also includes hydrophilic microcapsules surface modified with hydroxyl groups. Various agents such as estrone may be attached to the microcapsules and effectively targeted to selected organs.

DESCRIPTION

THERAPEUTIC AND DIAGNOSTIC USE
OF MODIFIED POLYMERIC MICROCAPSULES

5

BACKGROUND OF THE INVENTION

Field of the Invention

10   The invention relates generally to a reproducible, efficient method of preparing nonaggregated microcapsules. The microcapsules are suitable for encapsulation or conjugation with substances useful as diagnostic and therapeutic agents. The invention also
15   relates to amino-acid surface modified microcapsules and microcapsules conjugated with agents having particular potential for drug targeting.

Description of Related Art

20

        Microencapsulation is a well-studied art. It is basically the use of a matrix or encapsulating material to enclose gases, liquids or solids into particles of relatively small size (nanoparticles up to 500 μm). The
25   matrix is capsular material selected according to the intended use of the microcapsules.

        Physical properties of encapsulated chemical entities may be modified because of the encapsulation.
30   Other effects of encapsulation include dispersion of one substance within another, stabilization of emulsions and alteration of solubility rate. One of the most useful properties of encapsulated therapeutic materials is controlled release (Wright, *et al.*, 1989; Wright *et al.*,
35   1984).

Microcapsules have been prepared by many methods,
including coacervation, interfacial polymerization,
mechanical methods, polymer dispersion and matrix
encapsulation. Sustained release microcapsules have been
5   prepared from ethylcellulose (Kawashima, et al., 1984)
and poly-(D,L)-lactide (Benita, et al., 1984). There is
voluminous literature on the preparation and use of
encapsulating polymers designed for sustained drug
release (Bechtel, 1986; Tice, et al., 1989).
10

Although many preparations of microencapsulated
compounds have been reported, few describe microparticles
in the size range below 10 μm. Particles of 1-250 μm are
typically prepared by a solvent evaporation technique
15   (Tice and Gilley, 1985) while sizes from 1-10 μm have
been made by emulsion deposition (Smith and Hunneyball,
1986). One method using solvent evaporation claims to
provide a range of sizes from 0.5 - 250 μm (Mosier,
1985). Nevertheless, none of these methods appears to
20   provide a homogeneous preparation of single-particle,
nonaggregated microcapsules. Typical of these
preparations is a tendency to aggregate having an overall
size of about 177 to 395 μm with 5-162 μm particles
making up the aggregates (Jaffe, 1981). This technique
25   requires sieving to remove larger agglomerates, leaving
behind a wide range of particle sizes which, although
composed of small spheres, are nevertheless in aggregated
form.

30   Discrete microprills, polymeric particles in which a
drug (for example, Mellarib™) is uniformly dispersed,
have been disclosed (Fong, 1990). Although the
microprills were reported to be nonaggregated, the
average size range was 10-50 μm.
35

Lack of particle size homogeneity may cause severe
problems in quality control and in clinical use. For

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example, in chemoembolization studies, the particle
diameter is fairly critical in that only a limited range
of sizes will lodge in a target area (Bechtel, *et al.*,
1986). If too large, damage to larger vessels may occur,
5   while if too small, the particles pass through and drug
is not released at the targeted site. Thus a homogeneous
particle preparation is important.

       Despite the proliferation of microencapsulation
10   methods, there is a particular need for simple and
efficient methods of producing homogeneous preparations
of microencapsulated agents for clinical treatment and
diagnosis, most particularly in small, nonaggregated
particles ranging from 0.5 to 500 μm. A method of
15   preparing encapsulated therapeutic agents in 1 μm and 100
μm particles would provide more effective agents,
particularly for diagnostic imaging and
chemoembolization.

20   Bioimaging agents microencapsulated in 1 μm
particles would provide an ideal size particle for
bioimaging studies, particularly if combined with
capsular material selected to concentrate in the organ of
interest. Additionally, the use of microencapsulation
25   materials capable of targeting particular areas *in vivo*
would enable improvements in biodistribution imaging
studies as well as in drug delivery to specific organs.

SUMMARY OF THE INVENTION

30

       The present invention is a highly efficient,
reproducible method of obtaining homogeneous
nonaggregated preparations of polymeric microcapsules in
which therapeutic or diagnostic agents may be
35   encapsulated or conjugated. The invention also includes
microcapsules prepared from polymers conjugated to an
amino acid, enabling improved targeting of drug-laden

microcapsules to a particular target organ or cell. The
invention illustrates two important size ranges, 1 and
100 μm, of polymeric particles useful in clinical studies
and in which imaging or therapeutic agents may be
5   efficiently encapsulated.

An important aspect of the invention is the
preparation of homogeneous nonaggregated microcapsules
having a diameter of approximately 1 μm. These
10   microcapsules are prepared by combining a solution which
may contain a drug or therapeutic agent, a nontoxic
emulsifier and polymer dissolved in convenient solvent,
and then vigorously agitating the mixture. Agitation is
performed for a period of time sufficient for the
15   development of microcapsules having a mean diameter below
5 μm. The formation of the microcapsules is monitored
periodically, after which the organic solvent is removed
and the microcapsules collected.

20   The nontoxic emulsifier may be selected from several
commonly used emulsifiers, for example Tween-80,
polyvinyl alcohol, sodium laurylsulfate, Span 20, Lubrol,
Triton™ X-100, cetylpyridinium chloride and the like.
Thus a wide variety of emulsifiers may be suitable,
25   including anionic, cationic, and non-ionic types.

Likewise, a wide variety of materials may be used
for the preparation of the capsules, including
nonpolymers such as cholesterol, diglycerol, ethyl
30   cellulose as well as numerous types of polymers.
Microcapsules particularly useful for clinical or
therapeutic purposes release their contents by erosion,
degradation or diffusion. This is not to say that
microcapsule polymers used for medical treatment must be
35   biodegradable. For example, relatively permanent
implantable drug-containing polymers (e.g., hydrogels)
might be used for long-term sustained release in certain

applications. Polymers particularly suitable for
microencapsulation include poly-(D,L)-lactic acid,
ethylhydroxyethyl cellulose, polycaprolactone,
polycaprolactone diol, polylysine, polyglycolic acid,
5   poly-benzyl-L-glutamic acid, polymaleic acid and the
like.

Generally speaking, the emulsifier is soluble in
water, while the polymer, typically water insoluble, is
10 dissolved in an appropriate organic solvent. Water
immiscible or miscible organic solvents may be used,
depending on the nature of the polymer. Examples of
solvents include, but are not limited to, acetone, water,
ethyl acetate, chloroform, carbon tetrachloride and
15 methylene chloride.

An important step in the preparation of
nonaggregated microcapsules less than 5 μm in diameter is
the vigorous agitation of the mixture containing polymer,
20 emulsifier and, when desired, a diagnostic or therapeutic
agent. Agitation may be carried out by stirring,
sonication, or a combination of agitation methods. If
stirring alone is used, a speed of approximately 1500
rpms is preferred; however, where 1 μm preparations are
25 desired, it is preferable to use sonication alone or in
combination with stirring. If both stirring and
sonication are used, sonication at approximately 20 Khz
and stirring at 500 rpms are preferred settings.
Sonication and stirring are most preferably used
30 simultaneously. Agitation is continued for a period of
time sufficient to form individual microcapsules with an
average size less than 5 μm, typically at least 5 min and
more preferably 10 minutes. Under the general conditions
described, somewhat longer periods of time may be
35 required depending on the polymer, the organic solvent
used, the volume and concentration of starting material
as well as pH and temperature. Microcapsule formation is

typically monitored by periodically examining size and
shape of the microcapsules as they form in solution.
This step is particularly useful when optimizing time and
agitating conditions to assure homogeneous preparations

5   in the desired size range. Any method that detects size
and shape of the capsules may be used, for example,
removal of a drop of the solution and inspection under a
light microscope at a magnification of approximately 600
fold.

10
After the microcapsules have formed, the organic
solvent is removed from the mixture. A convenient
method, particularly for lower boiling organic solvents,
is to stir the reaction mixture at relatively slow rpms,

15   for example about 350 rpm, for a period of several hours
until the solvent is completely evaporated. The length
of time depends on the type and volume of solvent in
addition to other factors related to physical properties.
For example, the solvent acetone require about six hours

20   for complete evaporation. Other solvents with lower
vapor pressure/higher boiling points may require longer
periods of time. Evaporation, in this process, occurs at
room temperature, but higher temperatures may be applied
when different solvents are used. Monitoring of capsule

25   size and shape continues to be important throughout the
evaporation phase to assure that aggregation does not
occur.

The microcapsules are collected, after complete

30   evaporation of the organic solvent, preferably by
filtration, for example, by filtration through a nylon
mesh or other suitable filter that allows smaller
particles to pass through while retaining the larger
particles. The resulting suspension containing 1 μm

35   microcapsules may then be further processed to isolate
and store or use the particles. This is conveniently
accomplished by centrifuging the suspension after which

any residual organic solvent or emulsifier can be removed by washing either with water or sterile saline. The aqueous layer may then be decanted and the microcapsules resuspended in a liquid for storage or for therapeutic use. When used therapeutically, phosphate buffered saline, pH 7.4 is a most preferred resuspension medium. This method has provided a high yield (99%) of nonaggregated 1 μm particles. The amount of material collected in the nylon sieve is rarely over 1%, and the microparticles prepared by this method are remarkably uniform with a narrow size distribution ranging from 0.5 to 5.0 μm with the highest percentage being approximately 1.0 μm.

These microcapsules may be used to enhance or modify properties of diagnostic or therapeutic agents by virtue of the encapsulation. For example, in order to alter biodistribution properties, an ionic radiographic contrast agent may be encapsulated in a nonionic coat using this microencapsulation process. In the first step of preparing an encapsulated drug, the diagnostic or therapeutic agent is added to a mixture containing an aqueous solution, an emulsifier, and a polymer dissolved in solvent. During microparticle formation, the drug is encapsulated. The yield depends on the material being encapsulated. For example, 1 μm and 100 μm capsules of meglumine diatrizoate have relatively high efficiencies of encapsulation of 66% and 46% by weight respectively. Therapeutic agents (cisplatin, 5-fluorouracil and Tamoxifen) and diagnostic agents (Ethiodol, Iohexol, diatrizoate and Hexabrix) have also been incorporated into 100 μm capsules. Encapsulation is not intended to be limited to these particular drugs and it is envisioned that most therapeutic and diagnostic agents, whether water soluble or insoluble, could be encapsulated by this simple method.

Those skilled in the art will appreciate that this
method of encapsulation of therapeutic or diagnostic
agents will result in an entrapment of the material,
which will be released from the microcapsule at different
5   rates depending on the relative amount of polymer to
amount of drug encapsulated. Other factors affecting the
rate of release are the chemistry of the compound being
encapsulated, the environment into which the microcapsule
is being placed, temperature of the environment and the
10   nature or chemical composition of the capsular material.
The rate of release of drug will also be determined by
the relative ratios of drug to polymer, the type of
polymer, and the biodegradability of the polymer.

15   One μm microencapsulated imaging agents are ideal
for diagnostic imaging procedures and are readily
prepared by the method of the invention. First, a
homogeneous nonaggregated preparation of a 1 μm
microencapsulated imaging agent is prepared as previously
20   described. The material may be any standard imaging
agent, for example, an iodinated compound such as
meglumine diatrizoate. The microencapsulated imaging
agent can then be administered to an animal or human,
preferably by intra-arterial or intravenous injection.
25   The imaging agent is then detected by appropriate means
such as computed tomography or intravenous urography.

The general method used for the preparation of 1 μm
microcapsules can also be used to make microcapsules of
30   somewhat larger sizes, for example, 100 μm. Non-
aggregated microcapsules having a mean diameter of 100 μm
can be prepared by combining a polymer in a solvent with
a solution of a nontoxic emulsifier. The mixture is
emulsified by stirring at low speed, approximately 350
35   rpm, while monitoring microcapsule formation. The
solvent is then evaporated and the microcapsules
collected.

One difference between the procedure for preparing
100 μm microcapsules and preparing 1 μm microcapsules is
stirring the mixture of the polymer and the emulsifier at
a relatively lower speed when the larger particles are
5   desired. The stirring speed is approximately 350-400
rpm. During the stirring process, the size and shape of
the particles in the mixture are monitored, for example,
by using a light microscope at approximately 125x
magnification. After the desired size range of
10   microcapsules has formed, the organic solvent is removed,
preferably by evaporation and simultaneous stirring at
room temperature. After collection and drying, the
microcapsules are preferably sized. This may be
accomplished by passing the particles through various
15   sized filters, for example, first 600 μm mesh, then 600-
500 μm mesh, then 500-355 μm mesh, then 355-212 μm mesh,
and finally 106 μm mesh. The sieved particles yield a
mixture containing size ranges of approximately 106-212
μm. Use of these mesh sizes is for illustration purposes
20   only and, of course, any series of that same general size
mesh could be used. In the final step the 106-212 μm
particle mixture is sieved through a 106 μm mesh sieve
and the particles that pass through the sieve are
discarded. This provides a relatively uniform
25   preparation. Using this method, consistently
reproducible yields of approximately 70% of particle
sizes in the size range of 100-200 μm have been obtained.

In preparing 100 μm diameter particles, any of a
30   number of polymers may be used, including biodegradable
polymers such as poly-(D,L)-lactic acid,
ethylhydroxyethyl cellulose, polyhydroxybutyric acid,
polycaprolactone, polycaprolactone diol, polylysine,
polyglycolic acid or polymaleic acid,
35   polybenzylglutamate, polyhydroxypropylglutamate.

In the initial step of preparing 100 μm
microcapsules, a selected polymer is first dissolved in
an organic solvent then mixed with emulsifier. The
solvents may include methylene chloride, chloroform,
5   carbon tetrachloride, or other solvents in which the
polymer is soluble. The emulsifier may be selected from
any of a group of nonionic, cationic, or anionic
emulsifiers. A nontoxic emulsifier is preferably chosen
when the disclosed methods of microcapsule preparation
10   are used to encapsulate therapeutic or diagnostic agents
for *in vivo* use. The selected emulsifiers are preferably
solubilized in saline, although water or buffers may be
used. Hydrophobic or hydrophilic therapeutic or
diagnostic agents may be microencapsulated in the 100-200
15   μm particles. These compounds generally release slowly
from the microcapsules and the rate of release will
depend on the nature of the compound encapsulated, as
well as the type of polymeric material used for the
microcapsules.
20

It has been found that the 100-200 μm microcapsules
are ideal for chemoembolization. When chemoembolization
is desired, a drug encapsulated in a biodegradable or
nondegradable polymer is prepared. The microcapsule
25   generally has a diameter of about 100 μm which is
somewhat larger than the diameter of the tumor vessels in
the targeted organ. Encapsulated material is
administered intra-arterially causing occlusion of the
arteries. By occluding the arterial supply to neoplasms
30   with 100 μm capsules, the ischemia results in death of
the tumor cells. The rate of release will depend on the
nature of the material used to prepare the microcapsules.
Slow release over hours or weeks allows greater contact
time between the cytotoxic agents and tumor cell in an
35   anoxic environment which also increases capillary
permeability.

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Examples of microencapsulated drugs useful for chemoembolization are cisplatin, 5-fluorouracil and Tamoxifen. In one particular example, cisplatin was microencapsulated and administered into canine renal

5   arteries. Poly-(D,L)-lactide capsules and ethylhydroxyethyl cellulose capsules, both loaded with cisplatin, exhibited sustained cisplatin release for at least several days. The resulting tissue destruction was significantly greater than that with blank capsules.

10   These sustained-release properties should be similar for other drugs and other similar polymers.

Normally, drugs or other agents administered to an animal or human will initially disperse through the body

15   before concentrating in the liver, spleen, kidneys and urinary bladder prior to elimination. The inventors have discovered that amino acid ester conjugation to polymers affects the distribution and uptake of the encapsulated imaging material. In a particular example,

20   phenylalanine-conjugated polylactic acid was used to encapsulate meglumine diatrizoate. An animal injected with phenylalanine ester-conjugated encapsulated diatrizoate showed faster liver uptake than animals injected with nonconjugated polymer capsules. In the

25   former case, imaging was possible as early as sixty minutes after injection. Two hours post injection, the nonconjugated microencapsulated material showed both liver and kidney uptake as well as presence in the systemic circulation. In contrast, the conjugated

30   microencapsulated material was concentrated mainly in the liver with little material indicated in the general circulation at two hours post-injection. Both non-conjugated and amino acid-conjugated poly-(D,L)-lactide microencapsulated diatrizoate permitted computed

35   tomography imaging up to three days after administration. Neither material was seen in the liver five days post-administration. *In vitro* mouse liver cell culture

studies confirmed that conjugated microcapsules were
mainly taken up by hepatocytes whereas the nonconjugated
microcapsules were taken up by Kupffer cells.

5   Other amino acids conjugated with polymeric
encapsulating material are also expected to show
selective targeting of encapsulated drugs. Examples
include tyrosine, tryptophan, methionine and the like. A
selected amino acid may be covalently conjugated to a
10   polymeric material via an amide bond to link
phenylalanine with polylactic acid, for example. This is
conveniently performed by carbodiimide coupling
procedures well known to those experienced in the art;
such as reacting with dicyclohexylcarbodiimide in the
15   presence of hydroxysuccinimide. Covalent bonds need not
be limited to linkages involving the primary amine of the
amino acid but might, where desired, utilize a sulfur-
carbon bond between a sulfhydryl-containing amino acid
and the polymer. Furthermore, depending on the nature of
20   the functional groups on the polymer, other types of
linkages could be formed, for example, ether linkages.
Other conjugates are also envisioned; for example,
sugars, amino acids or derivatives of these compounds
could also be employed to surface-modify a microcapsule
25   polymer.

       Surface properties of 100 μm microcapsules may be
modified in the same manner as surface properties of the
1 μm microcapsules by conjugating with various amino
30   acids or other surface-modifying materials. In
chemoembolization, surface modification would likely be
important. These particles could be delivered intra-
arterially to the organ of interest.

35   In many situations, drugs or targeting agents may
be conjugated with a selected polymer prior to formation
of microcapsules. However, this is not be feasible for

some types of targeting agents, including many antibodies
or other compounds that might be altered during
microcapsule preparation. Such species may be conjugated
to surface groups of polymeric material in already formed
5   microcapsules.

Yet another aspect of the invention relates to
microcapsules modified by attachment of selected
targeting agents. Attachment is typically covalent, and
10   the nature of the chemical bond depends on the particular
polymer used to prepare the microcapsule. For example,
selected agents with amine functionalities may be reacted
with carboxyl moieties on a selected polymer using
coupling methods well-known to those of skill in the art.
15   Other modifications include for example, creation of
"spacers" on either the target molecule or groups on the
microcapsule polymer, although such spacer groups are not
necessarily required. In a preferred embodiment, poly
benzyl-L-glutamic acid polymer is conjugated with
20   estrone, an estrogen-receptor targeting compound. The
conjugated material may then be employed for tumor
targeting or imaging studies in organs high in estrogen
receptors. Such surface modification of·microcapsules
significantly alters tissue distribution, as demonstrated
25   in the higher uterus-to-muscle ratios achieved with
estrone conjugated ^{131}I-labeled microcapsules compared
with the labeled microcapsules alone.

A preferred polymer for conjugation of targeting
30   agents is poly-benzyl-L-glutamic acid. When desired
agents are attached to the polymer, an important
consideration is the percent of targeting material in the
conjugated product. While a high amount may appear
desirable, it has been found that substitution is
35   preferably limited to a degree that will permit
solubility in a solvent suitable for microcapsule
preparation by the disclosed solvent evaporation method.

This amount will vary with the nature of the polymer used and with the attached agent. As an example, 12% estrone content in estrone conjugated poly-benzyl-L-glutamic acid microcapsules exhibited good targeting properties while yielding a homogeneous, nonaggregated 1 μm preparation of microcapsules. At high concentrations, an attached targeting agent may adversely affect microcapsule formation.

While conjugation with estrone has been used to demonstrate the targeting properties of conjugated microcapsules prepared in accordance with the invention, it will be appreciated that enhanced targeting is associated with the microcapsules themselves. Thus, in general, the various polymeric microcapsules disclosed may be surface-conjugated with a wide variety of desirable targeting agents including, but not limited to, steroids, antibodies, particularly monoclonal antibodies or epitopic segments or fragments of antibodies, ricin A conjugated compounds, specific targeting drugs such as Tamoxifen, and the like. Further modifications may be made by attaching targeting agents to microcapsules modified with amino acid groups in accordance with preparations herein disclosed.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the *in vitro* profile of Tamoxifen from Tamoxifen microcapsules with Tamoxifen:polymer ratios of 1:1. A statistically significant difference from the corresponding sample after 1 hr of incubation (p<0.05 by Student T-test) was determined. Each bar represents the mean ± standard deviation of three samples.

Figure 2 shows the *in vitro* release rate profile of Tamoxifen from Tamoxifen microcapsules with

Tamoxifen:polymer ratios of 1:3. A statistically
significant difference from the corresponding sample
after 1 hr of incubation (p< 0.05, Student T-test) was
determined. Each bar represents the mean ± standard
5   deviation of three samples.

       Figure 3 shows the *in vitro* release rate profile of
5-fluorouracil from 5-fluorouracil microcapsules with 5-
fluorouracil:polymer ratios of 1:1. A statistically
10   significant difference from the corresponding sample
after 1 hr of incubation time (p< 0.05, Student T-test)
was determined. Each bar represents the mean ± standard
deviation of three samples.

15   Figure 4 shows the *in vitro* release rate profile of
5-fluorouracil microcapsules with 5-fluorouracil ratios
of 1:3. A statistically significant difference from the
corresponding sample after 1 hr of incubation (p<0.05,
Student T-test) was determined. Each bar represents the
20   mean ± standard deviation of three samples.

       Figure 5 is a scanning electron micrograph of PLA
microcapsules loaded with Tamoxifen with TX:PLA ratios of
1:1.
25
       Figure 6 is a scanning electron micrograph of PCL
microcapsules loaded with 5-fluorouracil:PCL ratios of
1:1.

30   Figure 7 is a scanning electron micrograph of 1 μm
PLA microcapsules (7B) and PLA microcapsules
encapsulating meglumine diatrizoate (7A) polymer Drug to
ratios were 1:3.

35   Figure 8 is a microcapsule size distribution curve
with data taken from Coulter Counter measurements.

Polylactic acid microcapsules were loaded with meglumine diatrizoate.

Figure 9 is a microcapsule size distribution curve prepared from data obtained from Coulter Counter measurements. The mean particle size for the PLA-PHE microcapsules loaded with diatrizoic acid is 3 μm with a range from 2-7 μm.

Figure 10 is a normal mouse hepatocyte culture shown under 40x magnification. All plates were seeded with aliquots from the same cell suspension. 10A shows control (no capsules) hepatocytes; 10B shows hepatocytes incubated for two hours with meglumine diatrizoate-loaded 1 μm poly-(D,L)-lactide capsules; Figure 10C shows hepatocytes incubated for two hours with meglumine diatrizoate-loaded 1 μm phenylalanine ester-conjugated poly-(D,L)-lactide capsules.

Figure 11 is a normal mouse Kupffer cell culture shown under 40x magnification. All plates were seeded with aliquots from the same cell suspension. Figure 11A shows control hepatocytes; 11B shows Kupffer cells after incubation for two hours with 1 μm poly-(D,L)-lactide capsules; 11C shows Kupffer after incubation for two hours with 1 μm phenylalanine ester-conjugated poly-(D,L)-lactide capsules.

Figure 12 shows a computerized tomographic images of two rabbits after intravenous injection with microencapsulated meglumine diatrizoate. Panels A-F show distribution of 1 μm poly-(D,L)-lactide capsules loaded with meglumine diatrizoate before (A) and immediately post-injection (B), 1 hr. (C), 2 hr. (D), 57 hr. (E), and 120 hr. (F). Panels G-L show distribution of the 1 μm phenylalanine-conjugated poly-(D,L)-lactide capsules loaded with meglumine diatrizoate before (G) and

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immediately post-injection (H), 1 hr. (I), 2 hr. (J), 57
hr. (K), and 120 hr. (L).

5   Figure 13 shows the release rate of CDDP from 100 μm
polylactide capsules as measured in jugular and renal
vein plasma in dogs at selected times over a period of 6
hours. The drug was administered intra-arterially into
the renal artery.

10   Figure 14 shows the release rate of CDDP from 100 μm
EHEC capsules as measured in jugular and renal vein
plasma from dogs at selected times over a period of 6
hours. The drug was administered intra-arterially into
the renal artery.
15

       Figure 15 schematically illustrates the coupling
reaction between estrone and poly-benzyl-L-glutamic acid.

       Figure 16 shows an estrogen receptor assay; Figure
20   16A is Scatchard analysis; 16B shows the saturation curve
for estrogen receptor binding assay in pig uteri.

       Figure 17 shows micrographs of cis-platin loaded
PBLG (poly benzyl-L-glutamate) microcapsules; Figure 17A
25   is a scanning electron micrograph of batch 1 cisplatin-
loaded PBLG capsules (100-200 μm), 29.2% (w/w) drug
loading, 1:1.5 drug-to-polymer ratio; 17B shows an
optical micrographs of cross-sections of PBLG 250 μ
microcapsules, 2:1 drug-to-polymer ratio, 37.5% drug
30   loading.

       Figure 18 shows the effect of drug loading after
viscosity changes in the organic phase on the cisplatin
release rate from cisplatin-PBLG microcapsules into
35   phosphate buffered saline (PBS) solution.

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Figure 19 shows the effect of drug loading on the rate of cisplatin release from cisplatin-PBLG microcapsules into PBS solution. Results for two different preparations (solid bar) and (hatched bar) are
5   shown.

Figure 20 shows the results of uptake of ^{18}F-labeled tamoxifen by pig uterus as measured by PET; Figure 20A is a radiograph of a pig's pelvic region. The bright site
10   is the uterus; Figure 20B is a PET image of the pelvic region of a pig receiving the ^{18}F-labelled tamoxifen; Figure 20C indicates that uptake in the uterus was blocked after pretreatment with estrone-PBLG (200 mg) empty particles. The image was obtained 1 hour after
15   injection of the ^{18}F-labelled tamoxifen.

Figure 21 shows the reaction scheme for the preparation of poly(hydroxylpropyl glutamate) and polyhydroxypropyl microspheres employing aminopropyl
20   alcohol modification of PBLG microspheres. Figure 21A shows the preparation of pHPG microspheres from PBLG. Figure 21B shows how PBLG microspheres can be reacted with tyramine aminopropylalcohol to form phenol conjugates which readily react with iodine. Labelled
25   microcapsules may be formed using Iodogen-I^{131}.

Figure 22 shows the distribution of polylactate microcapsules labeled with diatrizoate (panel A), phenylalanine conjugated polylactate microcapsules
30   labeled with diatrizoate (panel B) and diatrizoate (panel C), in blood, lung, liver, spleen and kidney after the indicated intervals.

Figure 23 compares liver/blood uptake ratio over a
35   period of approximately 6 hr for polyethylene glycol conjugated iopanoic acid (PEG-IOPA) and iopanoic acid (IOPA-E).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The invention is a method of preparing micro-
encapsulated therapeutic and diagnostic agents in
5   discrete nonaggregated particles suitable for diagnostic
radiologic studies and therapeutic use in humans. The
novel microcapsules of the invention are useful for
selective targeting *in vivo* because of the modified
surface characteristics. In one aspect, the invention is
10   the preparation of hydrophilic microcapsules to which a
wide variety of drugs may be attached and which target to
sites other than the liver. The method also relates to
the preparation of 1 μm particles for intravenous and
intra-arterial administration as well as 100 μm particles
15   for intra-arterial use. In other aspects of the
invention, cells in the body are specifically targeted
with drugs microencapsulated in polymeric material whose
surface properties are modified by conjugation with an
amino acid. The microcapsules may be conjugated or used
20   to encapsulate targeting agents which bind to specific
body cell receptors, including steroids, antibodies and
the like.

Materials and Methods

25

Poly(benzyl-L-glutamate) of two average molecular
weights (MW 58,000 and 43,000) was obtained from Sigma
Chemical Co. (St. Louis, MO). Poly-(D,L)-(lactic acid),
was obtained from Polysciences, Inc. (Warrington, PA).
30   Cisplatin and estrone were also supplied by Sigma as
powders of unspecified size. To prepare cisplatin-
containing microcapsules, the cisplatin crystals were
ground manually with a pestle in a mortar to an average
size of about 3 μm.

35

Polyvinyl alcohol (MW 30,000-70,000) was obtained
from Sigma and used as an emulsifier as received.

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Chempure™ methylene chloride solvent supplied by Curtin
Matheson Scientific, Inc. (Houston, TX), was used without
further purification. Iopanoic acid was purchased from
Sigma and converted to ethyliopanoate for radiolabeling.
5   Radiotracer: [^{131}I]sodium iodide (specific activity 7.75
Ci/mg, 680 mCi/ml) was obtained from Dupont New England
Nuclear (Boston, MA). Rats: Female rats weighing 100-
125 g were purchased from Harlan Sprague-Dawley, Inc.
(Indianapolis, IN).
10

Large Microcapsule (100-200 μm) Preparation

Drug-loaded capsules were produced by the solvent-
evaporation procedure according to Example 1. Various
15   amounts of cisplatin and poly(benzyl-L-glutamate) were
dispersed in methylene chloride depending on the drug-to-
polymer ratio desired. The cisplatin: PBLG ratios were
2:1 (0.8 g: 0.4 g), 1:1 (0.5 g:0.5 g), 1:1.5 (0.33 g:0.5
g), and 1:2 (0.4 g:0.8 g). The cisplatin crystals were
20   ground with a mortar and pestle for 5 minutes before
being weighed. The appropriate amounts of drug and
polymer were then stirred for 20 minutes or more in 5-20
ml of methylene chloride. This organic phase was
emulsified in 250 ml of water containing 2% (w/v)
25   polyvinyl alcohol spun at 350 rpm. The resulting mixture
was stirred for 5 hours at room temperature (24°C) to
ensure complete evaporation of the solvent. The contents
of the beaker were then poured through a Buchner funnel
under suction. The microcapsules remaining on the filter
30   paper were washed with 250 ml of water to remove the
emulsifier. Cisplatin crystals were left on the filter to
air dry. Microcapsules were collected using a sieve to
separate the 100-200 μm fraction.

Determination of Cisplatin Content

Ten milligrams of each batch of capsules was
dissolved in 5 ml of N,N-dimethylformamide (Fisher
5   Scientific Co., Fiar Law, NJ). The amount of cisplatin
in the resulting solution was determined using a Perkin
Elmer model 55 ultraviolet spectrophotometer (Coleman
Instruments Division, Oakbrook, IL) at 310 nm. A
standard curve was produced using the same procedure by
10   adding a known amount of pure cisplatin (5 mg).
Experiments were performed in triplicate. The drug
content was calculated as a percentage of the total
weight of the capsule.

15   *In Vitro* Sustained Release

Because of variations in the yield of each batch of
capsules after sieving, release rates were run in
triplicate. Thirty milligrams of capsules were weighted
20   into VACUTAINER brand evacuated blood collection tubes,
10 ml draw (Becton Dickinson VACUTAINER Systems,
Rutherfored, NJ), and 5 ml of Dulbecco's phosphate
buffered saline (PBS) without calcium or magnesium (Sigma
Chemical Co.) was added. Initially, the tubes were
25   inverted several times to ensure contact of the capsules
to the PBS (pH 7.4). The test tubes were immersed in a
water bath at 37°C and shaken on a water bath.

Tubes were periodically centrifuged at 2500 rpms for
30   5 minutes and 3 ml of the PBS drawn off and analyzed
using ultraviolet spectrophotometry. The remaining 2 ml
of solution was removed and 5 ml of fresh PBS added for
each measurement. Tubes were inverted several times
before returning to the shaker bath. The effects of
35   centrifugation on capsule morphology were examined using
cross-sections of capsules centrifuged for six 5-minute
intervals.

Microscopy Studies

        Surface characteristics of the microcapsules were
evaluated using a scanning electron microscope. One
5   micrometer cross sections of the capsules were obtained
and embedded in EPON, a Medcast resin (Ted Pella, Inc.,
Redding, CA), cast in BEEM imbedding capsules (Ted
Pella), and cut on a microtome. Cross sections were
photographed with an Axiovert 405M inverted
10   photomicroscope (Zeiss, Germany) equipped with a long
distance condenser for differential-interference contrast
and a 35 mm camera.

Radiolabeling of Ethyl Iopanoate

15

        2 g (3.5 mmol) of iopanoic acid was dissolved in 50
ml absolute ethanol, and 0.4 ml (5,25 mmol) thionyl
chloride was added. The reaction was refluxed for 3
hours. After cooling, the reaction mixture was
20   evaporated and reconstituted in 100 ml methylene
chloride. The organic mixture was washed twice with 25
ml 5% NaOH and twice with 25 ml water. The methylene
chloride layer was dried over $MgSO_4$ and evaporated to
dryness, yielding 1.73 g of ethyliopanoate (82.4%). The
25   structure was provided by ^1H nuclear magnetic resonance
and mass spectrometry (M^+ 599). The radioisotope
exchange reaction was carried out using a known procedure
with some modification (Zupon, et al., 1983; Kroschwitz,
1989). Briefly, 10 mg of the ester and 0.3 ml of
30   tetrahydrofuran were placed in a vial and treated with
1.6 mCi of $[^{131}I]$ sodium iodide (in 100 μl of 0.1 M sodium
borate buffer). Pivalic acid (25 mg) was then added.
The reaction vial was sealed and heated at 150°C for 1.5
hours. The vial was cooled and the ethyliopanoate
35   reconstituted in methylene chloride (0.1 ml) and
chromatographed on a silica gel column with methylene
chloride/methanol (9:1) as the eluent. This yielded 0.54

mCi ethyliopanoate (34%). Radiochemical purity was
determined by co-chromatography on a silica gel plate
eluted with methylene chloride methanol (9:1); unlabeled
ester served as the standard, with a retardation factor
5   of 0.80.

In Vivo Tissue Distribution

      PBLG and estrone-PBLG microcapsules loaded with
10   [^{131}I]ethyliopanoate (5.7 μCi in 0.6 ml of water) were
administered to rats in the tail vein. Rats (N=3/group)
were sacrificed at 1, 3, 6, and 24 hours after injection.
The percentage of injected dose in an organ or tissue was
determined by a gamma counter.
15

Positron Emission Tomographic Evaluation of PBLG
Microcapsules

      Positron emission tomography (PET) imaging was
20   performed on four domestic female pigs (30 lb) with a
positron camera (Positron Corp., Houston, TX). A 20-
minute attenuation scan was performed with a 4-mCi ^{68}Ge-
ring source prior to tracer injection. After each pig
received 10 mCi of ^{18}F-labelled tamoxifen, eight
25   consecutive 10-minute scans were performed employing a 5-
minute wait between scans for data transfer. Total
counts collected per scan was 15-30 million. Serial
transaxial images of the pelvic region enabled viewing of
the uterus. The tomograph has a field of view of 42 cm
30   on the transverse plane and 12 cm on the coronal plane.
The axial resolution on the reconstructed plane is 1.2
cm. Twenty-one transaxial slices separated by 5.2 mm
were reconstructed for each scan.

35   Each pig was supine in the scanner to allow the
detector rings to span the entire pelvic region. Prior
to scanning, the position of the uterus and ovaries was

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determined by hysterosalpingography. Fifteen milliliters
of radiopaque (Renografin 76, Squibb Diagnostic, New
Brunswick, NJ) was injected through the vagina into the
uterus through a 5 Fr catheter whose balloon was inflated
5   by 1 ml of air. Radiographs of the pelvis in the
anterior-posterior position were taken. The location of
the uterus was marked permanently on the skin of each pig
for consistent positioning in the PET camera. The same
positioning was used in subsequent scanning.

10

To demonstrate that the estrone-PBLG uptake in the
uterus and ovaries was effected by estrogen receptors, a
pig was given estrone-PBLG (200 mg) empty capsules 30
minutes before intravenous injection of the $[^{18}F]$-labelled
15   tamoxifen (Yang, *et al.*, 1991)

Morphology and Release Pattern of Large Microcapsules

Cisplatin-containing capsules of 100-200 μm prepared
20   by the process described herein were appropriate for *in
vivo* use as determined by gas chromatography, mass
spectrometry with a mass selective detector. The amount
of residual methylene chloride in the capsules was less
than 0.2 ppm.

25

Scanning electron microscopy showed that almost all
the drug was encapsulated, regardless of the drug-to-
polymer ratio. All the capsules had porous outer
surfaces (Figures 17A and 17B). Processing conditions
30   and experimental loading yields are given in Table 1.
The efficiency of drug loading in the microcapsule
prepared is clearly influenced by the viscosity of the
organic phase.

35   The factors affecting drug loading also direct the
release rates of microcapsules. Figure 18 indicates that
higher loading due to increased viscosity of the organic

phase causes capsules to release their drug load more
slowly. Microcapsules with a 21.53% drug load (20 ml
CH_2Cl_2) exhibited a strong initial release and continued
to release rapidly for the first 24 hours. At 43.96%
5   loading, the microcapsules prepared with 5 ml of
methylene chloride released in a slower, more linear
fashion and did not reach a release plateau until after
96 hours. The difference between the two release rates
was especially striking during the first hour. The lower
10   loaded capsule (21.53%) released 26.0% of its load within
the first hour of being introduced to the PBS; however,
the capsule bearing 43.96% drug lost only 5.8% of its
drug load under the same conditions.

15   As seen in Figure 19, the capsule with higher
loading resulting from a higher core-to-wall ratio, also
released more slowly, even though the amounts of
methylene chloride used in preparation of the capsules
were the same. All capsules displayed the same general
20   release pattern: an immediate strong release that tapered
off within the first 1 to 4 days. The loading affected
only the strength and duration of the initial release.
In no case was there any indication of degradation of the
polymer matrix such as would be indicated by a delay of
25   several days before drug release or a sudden increase in
drug release.

The processing conditions described herein yield
PBLG-cisplatin microcapsules in which higher drug loading
30   corresponds to more central drug concentration and slower
release rates. However, all capsules prepared
demonstrated sustained-release properties during 31 days
of monitoring without an initial or final burst that
would complicate their clinical use as a means of steady
35   drug administration.

*In Vitro Estrogen Receptor Assay of Estrone-PBLG
Conjugates*

Scatchard analysis of [³H]estradiol binding in pig
uteri indicated a single class of binding sites with a
mean binding affinity constant (kd) of 2.2 nM and a mean
receptor density (ßmax) of 350 fmol/mg protein. The
protein concentration used was 1 mg/ml cytosol. Hill
analysis (coefficient 0.992) indicated that estradiol has
competitive reversible binding. The IC_{50} for estrone was
5×10^{-8} M and for estrone-PBLG was 5×10^{-7} M (based 12%
conjugation).

In Vivo Tissue Distribution of Small Microcapsules

The results of tissue distribution studies for ^{131}I-
labeled microcapsule groups are shown in Tables 2 and 3.
The uterus-to-muscle radioactivity-uptake ratio in the
estrone-PBLG group was higher than that of the PBLG
group.

Underline PET Studies of Small Microcapsules

The PET image was correlated with the findings on
the hysterosalpingogram. Figure 20A is the transaxial
view of a PET image of the pelvis of a pig 1 hour after
administration of ^{18}F-labeled tamoxifen. The pig was
scanned in a caudal-to-cranial direction. Slices 2-6
showed increased tamoxifen uptake in the uterus and
ovaries (Figure 20B). This increased uptake was blocked
by pretreatment with estrone-PBLG (200 mg) empty capsules
(Figure 20C). Slices 2-6 show the effect of this
blockage. Here, the pig was scanned in a cranial-to-
caudal direction. The PET data indicate that the uptake
of estrone-PBLG microcapsules in the uterus and ovaries
was mediated by means of estrogen receptors.

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The following examples are intended to illustrate specific embodiments of the present invention. Those skilled in this field will recognize that modifications could be made to the disclosed methods and that other applications would remain within the scope of the present invention.

EXAMPLE 1

100 Micron Microcapsule Preparation of Microencapsulated Meglumine Diatrizoate

Meglumine diatrizoate, 2 g, was dispersed in 40 ml methylene chloride and 1 g poly-(D,L)-lactic acid added to the mixture. Encapsulation was achieved while stirring at 350 rpm in 250 ml 0.9% (w/v) saline solution containing 1.25 g polyvinyl alcohol. The pH of the solution was adjusted below 4 with 1 N HCl. From time to time, formation of microcapsules was determined by examining a drop of the material at 125x magnification under a light microscope. The mixture was stirred for approximately 6 hr until the methylene chloride was completely evaporated. The microcapsules were collected by filtration and washed with distilled water (2 x 100 ml). The microcapsules were air dried at room temperature and then sieved through various meshes, including stepwise, 600 μm mesh, 600-500 μm mesh, 500-355 μm mesh, 355-212 μm mesh and 106 μm mesh, to give a mixture containing particles of size range 106-212 μm. The weight of the 106-212 μm particles was approximately 70% of the initial total amount of the contrast agent plus polymer. The microcapsules contained 46% (w/w) of meglumine diatrizoate.

EXAMPLE 2

1 Micron Microcapsule Preparation of Microencapsulated
Meglumine Diatrizoate

All the following steps were done under aseptic
conditions using ultraviolet light with sterile
instrumentation.

Meglumine diatrizoate, 1.2 g (Sigma Chemical
Company, St Louis, MO), was dissolved in 100 ml water and
then 1 ml of Tween 80 was added. The mixture was stirred
at 500 rpm and the pH of the solution adjusted below 4
with 1 N HCl. To this mixture was added dropwise 0.5 g
poly-(D,L)-lactic acid (MW 30,000-60,000) dissolved in 10
ml acetone. The mixture was stirred at 1500 rpm or
sonicated at 20 Khz for 10 min and periodically monitored
under a light microscope at 600x magnification until
round particles of approximately 1 μm in diameter were
observed. The mixture was stirred at 1500 rpm (without
sonication) or 500 rpm (with sonication) for an
additional 6 hr or until the acetone was completely
evaporated. The microcapsules were collected by sieving
through a nylon mesh to remove a small amount of
aggregated material, approximately 1%. The microcapsule
suspension was centrifuged at 24,000g and washed 3 times
with saline to remove the emulsifier. The microcapsules
were resuspended in sterile phosphate buffered saline.
The microcapsules weighed 1.5 g (90% by total initial
weight of contrast plus polymer). The microcapsules
contained 66% by weight of meglumine diatrizoate. The
particles were cultured and found to be sterile.
Scanning electron microscopy (SEM) revealed round,
uniform particles as shown in Figure 7. The distribution
of particles was determined using a Coulter counter,
indicating a narrow range of 2-7 μm with 50% having a

mean capsular size less than 5 μm, as indicated in Figure
8.

EXAMPLE 3

5

Conjugation of Amino Acid Ester to Polylactic Acid

        To a solution of 2.0 g (0.05 mmol) poly-(D,L)-lactic
        acid in 10 ml dimethylformamide (DMF) was added 1.2 g
10   (5.5 mmol) of dicyclohexylcarbodiimide and 0.68 g (5.5
        mmol) of N-hydroxysuccinimide. After stirring 10 min,
        1.2 g (5 mmol) phenylalanine ester dissolved in 5 ml DMF
        was added. The mixture was stirred overnight. The solid
        urea was filtered. The filtrate was poured into 100 ml
15   water and the white solid precipitated. The solid was
        filtered, washed with 100 ml water, air dried and weighed
        to obtain 2.4 g (75%) of the total chemical yield. Thin
        layer chromatography indicated a single spot (Rf= 0.3,
        chloroform/methanol 9:1). The phenylalanine content in
20   the polymer conjugate was 23% as determined by
        ultraviolet spectroscopy at 254 nm. Similar conditions
        were used to prepare microcapsules of poly-(D,L)-lactic
        acid conjugated with methionine, tyrosine or tryptophan
        ester.

25

EXAMPLE 4

Chemoembolization with Microencapsulated Cisplatin

30   Eighteen adult mongrel dogs were anesthetized with
        intravenous sodium pentobarbital (Nembutal; Abbott, North
        Chicago, IL), 30 mg/kg, and an intravenous drip of normal
        saline was initiated. Through a cutdown, a 5-F
        polyethylene catheter was introduced into the femoral
35   artery, and the animal was given an intra-arterial bolus
        of sodium heparin (100 units/kg). The catheter was then
        advanced into one of the renal arteries. The ipsilateral

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renal vein was also catheterized via a femoral vein with
a 5-F catheter to sample blood for cisplatin (CDDP),
while simultaneous systemic venous blood samples were
collected through an 18-gauge Cathlon™ catheter inserted
5   in a jugular vein.

Microcapsules with an average size of 106 μm (range
50-350 μm) and containing cisplatin (40-43%) by weight,
were formulated as described in Example 1 from lactic
10   acid polymer and ethylhydroxyethyl cellulose polymer.
The capsules, in dry form, were sterilized with ethylene
oxide. The microcapsules were suspended in a 1:1
solution of radiographic contrast material. Iohexol
(Omnipaque, Nycombed, Norway) and normal saline such that
15   the final concentration was 20 mg/ml. The suspension was
administered into the renal artery until stasis of flow
was observed fluoroscopically. One kidney was embolized
in each of three animals with each of the capsular
materials containing CDDP, and one kidney from each of
20   five dogs was occluded with each of the capsular
materials without CDDP. Renal and systemic venous blood
samples were collected in heparinized tubes at 30-minute
intervals for 6 hours after embolization. The plasma was
analyzed for CDDP using atomic absorption. Drug release
25   curves were generated from these data. Two such curves
are shown in Figures 13 and 14. To evaluate renal and
hepatotoxicity, systemic venous blood samples were
collected before and at 1, 2, 3, 4, and 6 weeks after
embolization to determine blood urea nitrogen (BUN),
30   creatinine, and serum glutamic oxaloacetic transaminase
(SGPT) levels.

Renal angiography was performed with Omnipaque
before and immediately after embolization, at hourly
35   intervals up to 6 hours after embolization, and 1, 2, 4
and 6 weeks later to document the radiographic changes in
the occluded kidneys. After 6 weeks, each animal was

killed with an overdose of sodium pentobarbital, and a
complete necropsy performed. The gross and microscopic
findings in each dog were compared.

5   Both PLA and EHEC capsules without encapsulated drug
produced embolic effects in the kidneys. The polymers
loaded with cisplatin damaged kidneys significantly more
than polymers alone. PLA capsules loaded with cisplatin
had a greater effect on tissue than cisplatin-loaded EHEC
10   capsules. EHEC capsules without CDDP showed slightly
more degradation than PLA capsules in these studies.

     In vitro drug release data were also determined by
incubation of the microcapsules in phosphate buffered
15   saline. The data are shown in Table 1 for release of
CDDP from CDDP:PLA microcapsules.

TABLE 1

RELEASE RATE OF CDDP FROM CDDP MICROCAPSULES[1]
(SIZE 100 μm)

Incubation Time (min)	% Released
1	11.6
5	21.3
15	27.4
30	39.5
60	37.7
120	35.0
240	40.4

[1] CDDP:PLA = 1:1

EXAMPLE 5

Biodistribution of 1μ PLA Surface Moified Microcapsules

5   1 μm microcapsules loaded with meglumine diatrizoate
were prepared as described in Examples 2 and 3 using PLA
and PLA conjugated with phenylalanine (PLA-PHE) as the
capsular material. Each preparation was injected
intravenously into a rabbit and thereafter monitored by
10   computed tomography for organ uptake. The rabbit
receiving PLA-PHE showed a faster liver uptake than the
rabbit receiving PLA encapsulated diatrizoate. After 2
hr, the PLA-PHE treated rabbit showed liver uptake and
little, if any, contrast in the general circulation while
15   the PLA treated rabbit showed both liver uptake and
presence in the general circulation. After 48 and 72 hr,
both rabbits showed significant liver uptake.
Biodistribution is shown in Figure 22 which compares
tissue distribution of diatrizoate (DZ), ^{131}I-DZ labeled
20   polylactite (PLA) microcapsules and ^{131}I-DZ labeled
phenylalamine surface modified (PLA-PHE) microcapsules.

         The mean particle size of the PLA-PHE microcapsules
loaded with meglumine diatrizoate was determined to be 3
25   μm, as indicated from a particle size distribution curve
obtained using a Coulter Counter, Figure 9.

         In a second series of animal experiments, male
Webster mice (25-30g) were intravenously injected with
30   1μC; radiolabeled microspheres, then sacrifieced at 30
min, 1h, 3h, 6h and 24h. Organs were excised, weighed
and counted for radioactivity. The microcapsules
exhibited sustained release. Liver uptake was faster in
mice injected with labeled ple-modified capsules than in
35   mice receiving labeled unmodified capsules.

EXAMPLE 6

In Vitro Release Rates of 100 μm Microcapsules

5   Microcapsules were prepared as described in Example
1 using the solvent evaporation method with drug:polymer
ratios of 1:1 and 1:3 (w/w) and polyvinyl alcohol as
emulsifier. The biodegradable polymers used were PCL,
PCLD and polylactic acid (PLA). The cytotoxic compounds
10   Tamoxifen and 5-fluorouracil were dissolved in methylene
chloride, then added with the emulsifier to a water
solution with stirring at 400 rpm. After 6 hr, the
capsules were washed with water and air dried. Capsules
of approximately 100 μm were collected from mesh screens.
15   Assays on the encapsulated drugs were performed by
dissolving 5 mg of the microcapsules in 5 ml methanol.
The solution was centrifuged and 100 μl of the
supernatant diluted with 3 ml methanol and analyzed
spectrophotometrically at 238 nm. A standard solubility
20   time curve was produced using the same procedure by
adding 2 mg of both TX and 5-FU. The drug content was
calculated as a percent of total capsule weight.
Triplicate determinations were made.

25   Dissolution studies were performed on the
microencapsulated drugs. Capped test tubes were filled
with 5 ml of 0.05 M phosphate buffered saline pH 7.4 and
placed in a water bath shaker set at 100 rpm at 37°C. 5
mg of microcapsules were added to each test tube, and
30   sample solutions of 3 ml were collected at different time
intervals after centrifugation. After each
determination, the sample solutions were returned to each
test tube. The concentrations of the drug released from
microcapsules were determined by comparison with the
35   standard drug (2 mg) in the same dissolution solution for
the controls and measured spectrophotometrically at 238
nm. Determinations were made in triplicate. A Student's

T-test was used to compare the sample after 1 hr of
incubation and the corresponding sample at different
incubation time intervals (p< 0.05 level).

5   The percent of drug content in the various
biodegradable microcapsules is shown in Table 2 below.
Scanning electron microscopy showed that all the
microcapsules prepared were spherically shaped with
smooth outer surfaces (Figures 5 and 6).

10

TABLE 2

	% (W/W) DRUG IN MICROCAPSULES		
DRUG	POLYMER	DRUG:POLYMER	
		1:1	1:3
	PLA	30.0	22.5
Tamoxifen	PCL	30.7	13.0
	PCLD	36.4	14.9
	PLA	8.8	8.5
5-fluorouracil	PCL	9.9	6.6
	PCLD	7.6	7.6

Release rate of TX and 5-FU is shown in Figures 1
and 2. The release rate of TX (1:1 ratio) at 48 hr
incubation time decreased in the order: PLA>PCL>PCLD;
30   however, the release rate of 5-FU (1:1 and 1:3 ratios) at
48 hr incubation showed PCL>PCLD>PLA. This study
indicates that different polymers alter drug release
rate.

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EXAMPLE 7

Preparation of PHPG Microspheres

5   Poly(benzyl L-glutamate)(PBLG, Sigman) microspheres
were prepared by a solvent evaporation method according
to Example 1. Polybenzyl-L-glutamate (PBLG, 0.7 g) and
unlabeled ethyliopanoate (0.3 g) were dissolved in
methylene chloride (30 ml). To this mixture
10   [^{131}I]ethyliopanoate (320 μCi) was added. The organic
phase was emulsified in a water solution (200 ml)
containing polyvinyl alcohol (1% w/v). The mixture was
stirred at 2000 rpm for 25 hours to ensure complete
evaporation of the solvent. The suspension was then
15   centrifuged (12,000 rpm) for 10 minutes. The
microcapsules were separated, washed with water to remove
any excess polyvinyl alcohol and centrifuged again. The
resulting microcapsules were filtered through nylon cloth
(5-μm mesh). The final concentration was 154 μCi in 18
20   ml of water.
In a typical run, particles had a mean diameter of 2.0 μm
and over 95% of particles were less than 5 μm.

          PBLG microspheres were converted to PHPG hydrogel
25   microspheres by treating PBLG with aminopropyl alcohol
containing 3% of diaminohexane as a crosslinker at 70°C
for 2,3 or 5 hrs. To determine the extent of conversion,
PHPG microspheres were completely hydrolyzed and the
unsubstituted benzyl groups analyzed by HPLC.
30

          Figure 21 illustrates the conversion of poly(benzyl
L-glutamate) to poly(hydroxypropyl L-glutamine). A
schematic representation of the microspheres formed from
the polymer is also shown.

35

Radiolabeling of Microsphere

Microspheres were labeled with covalently bound ^{131}I
uprepared by treating PBLG microspheres with aminopropyl
5   alcohol in the presence of tyramine (1% w/w) followed by
Iodogen labeling (Wallace, *et al.*, 1988). Radiochemical
yield:65% with purity >95%.

In Vitro Stability Assay

10
^{131}I labeled PHPG particles (20 μCi/mL) were
incubated in 50% serum at 37°C. At various time
intervals, aliquots of serum were removed and
centrifuged. The radioactivity of supernatant was
15   measured with a γ-counter.

Organ Distribution of Microspheres

Female Sprague-Dawley rats (140-160 g) were
20   anesthetized with ketamine (10 mg, i.p.) and radiolabeled
microspheres were given i.v. (0.4 mL). The dose
corresponded to 12 mg dry microspheres with total
activity of 6±2 μCi. The animals were sacrificed at 20
min, 3, 6, 24, 48 and 96 hrs. The organs were excised,
25   weighed and counted for radioactivity.

Electron Microscopy Studies

Liver tissue samples were examined by TEM. 30 min
30   after administration of microspheres, liver was perfused
with 2% glutaraldehyde in 0.2 M sodium cacodylate buffer
through the portal vein. Tissue samples were processed
and stained using standard TEM methods. SEM of air dried
microspheres were examined in a Hitachi model S520
35   electron microscope.

PHPG microspheres were prepared directly from PBLG
microspheres by aminolysis. This approach made it
possible to prepare a series of PHPB microspheres with
different surface characteristics. The resulting
5   microspheres became increasingly hydrophilic with longer
reaction times. Swelling ratio increased from 3% (PBLG
microspheres) to 36% (PHPG microspheres) after 4 hrs
treatment with aminopropyl alcohol. The hydrophilicity
of PHPG microspheres was also evidenced by SEM which
10   showed that the microspheres tend to become flat after
drying in air.

Only 1% of radioactivity dissociated from PHPG
microspheres after incubation in 50% serum for 2 hrs. 96%
15   of radioactivity was found bound to PHPG microspheres
even after two days. Figure 18 shows the deposition of
three preparations of PHPG microspheres in liver, blood
and spleen 20 minutes post-administration. With the
increased hydrophilicity of the microspheres, there was a
20   substantial decreased uptake in liver; the concentration
of microspheres circulating in the blood was increased.
This indicated decreased uptake of microspheres in liver
Kupffer cells achieved by modification of PHPG
microspheres. Electron microscopic studies revealed that
25   PBLG microspheres were taken up by Kupffer cells. On the
other hand, no PHPG microspheres could be identified in
the Kupffer cells of rats.

                          EXAMPLE 8

3.0

The following example illustrates modification of
the phenolic group of estrone to enable coupling with
poly-benzyl-L-glutamate. The product illustrates a
"spacer" between the estrone 3-position functionality and
35   the conjugating amide bond.

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Preparation of 3-Aminoethyl Estrone

Estrone (5.0 g, 18.5 mmole) was dissolved in 80 mL
of anhydrous DMF. Sodium hydride (4.4 g, 185 mmole) was
5   slowly added to the solution to generate reactive
phenoxide in situ. Care was taken to avoid rapid
evolution of hydrogen gas. 4.3 g (55 mmole)
chloroethylamine was added to the solution and the
mixture was allowed to react at 60°C for 4 hrs. The
10   product was precipitated with a large volume of water and
the precipitate collected. For purification, the crude
solid was dissolved in methylene chloride, and washed
with water. Evaporation of methylene chloride yielded 3-
aminoethyl estrone which after washing with ethyl ether
15   gave 3.0 g (52%) of product m.p. 140°C (decomp.), 3-
aminoethyl estrone hydrochloride, m.p. 180°C (decomp.),
^{1}HNMR (ppm): δ3.01 (2,t,CH$_2$CH$_2$NH$_2$), 2.78 (2,t,CH$_2$,CH$_2$NH$_2$),
4.00 (2,t,COCH$_2$).

20   Coupling of 3-Aminoethyl Estrone to Poly(benzyl L-
glutamate) (PBLG)

The reaction below was conducted in p-dioxane as
solvent. The reaction may also be conducted in dimethyl
25   sulfoxide or dimethyl formamide with comparable success;
however these solvents are not so readily removed and are
therefore less preferable.

3-Aminoethyl estrone (1.25 g, 4 mmole) was added to
30   a 7 mL dioxane solution of PBLG (0.88 g, 4 mmole). The
mixture was allowed to react at 60°C for 2 days. The
conjugate formed was collected by precipitating the
dioxane solution with water, followed by filtration. For
purification, the solid was dissolved in methylene
35   chloride. Insoluble impurities were removed by
filtration. The methylene chloride solution was washed
with cold aqueous 0.2 N hydrochloric acid solution (x2),

water, and saturated NaCl until neutral. Evaporation of
methylene chloride yielded 0.4 g product. Elemental
analysis for the conjugate, calculated, C: 70.73; H:
7.60; N: 6.60; found, C: 66.70; H: 6.45; N: 6.00. Degree
of substitution was calculated to be 12% based on
elemental analysis data. ^{1}HNMR (ppm): $\delta 3.70$
(2,t,CH$_2$CH$_2$NHCO), 2.86 (2,t,CH$_2$CH$_2$NHCO), 4.04 (2,t,COCH$_2$).

Estrone-conjugated poly-benzyl-L-glutamate was
dissolved in p-dioxane and used to prepare 1 μm
microcapsules by the method of Example 2.

EXAMPLE 9

This example illustrates determination of binding
affinity constants for estradiol in pig uterus.

In Vitro Estrogen Receptor Assay

Affinity for binding the estrogen receptor was
determined.
30 g uteri obtained from domestic swine (30 kg) were
homogenized in 80ml of 10 mM Tris buffer, pH 7.4,
containing 1.5 mM EDTA and 3 mM sodium azide. The
homogenate was centrifuged at 1,000 x g for 1 hr at 4°C.
Uteri cytosol was then pretreated with dextran-coated
charcoal. To investigate the nature of estradiol
interaction with the estrogen receptor site, a saturation
curve was obtained from [^{3}H]estradiol (10^{-5} M to
10^{-10} M) in the presence or absence of excess estradiol
(10^{-5} M)(Figure 16). Uteri cytosol was incubated at 4°C
for 2 hr with [^{3}H]estradiol (5 nM/tube) and competitor
(ranging from 10^{-3} M to 10^{-8} M) or with estradiol (10^{-5}
M)(non-specific). The concentration of test compounds
that decreased specific radioligand binding by 50% (IC$_{50}$)
was measured. Protein concentrations were determined
according to the method of Lowry *et al.* (1956).

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Scatchard analysis indicated a single class of
binding sites with a mean binding affinity constant kd of
2.2 nM (n=9) and a mean receptor density (B max) of 350
fmol/mg protein, Figure 16. The protein concentration
5   used was 1 mg/ml cytosol. Hill analysis (0.992)
indicated that estradiol had competitive reversible
binding. The IC_{50} of estrone conjugates to poly-benzyl-L-
glutamate was 5×10^{-7} M which is ten-fold lower than the
binding affinity for estrone (5×10^{-8} M), Table 3.

10

TABLE 3

Comparison of EST-PG and Estrone on
15   Estrogen Receptor Binding in Pig Uterus

	$IC_{50}(M)$	Equiv. (Wt)
Estrone	5×10^{-8}	0.14 ng
EST-PG[a]	5×10^{-7}	500 ng

20

[a] EST-PG: Estrone with spacer (ethanolamine)
conjugates to polybenzylglutamate (MW 58,000)
[b] Based upon 12.0% of conjugation between estrone and
polymer, determined by UV at 282 nm and elemental
25   analysis.

EXAMPLE 10

This example provides data comparing percent tissue
30   uptake of estrone loaded polybenzyl-L-glutamate
microcapsules containing ^{131}I-iopanoate with microcapsules
containing ^{131}I-iopanoate but lacking estrone. Of
significance is the greater uptake of the estrone loaded
microcapsules by the uterus whereas there is less
35   relative uptake by microcapsules containing only the
labeling agent.

In Vivo Tissue Uptake

Estrone conjugated poly-benzyl-L-glutamate
microcapsules loaded with ^{131}I-labeled ethyliopanoate were
5   injected into rats (three per group) via the tail-vein
(5.7 μCi in 0.3 ml water). Control groups were given
only the ^{131}I-labeled iopanoic acid. Rats were sacrificed
at 1, 3, 6 and 24 hours post injection. The percent of
injected dose per organ or per tissue weight was
10   determined by a COBRA Auto-gamma counter (Packard,
Meridien, CT). Results are shown in Tables 4 and 5.

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TABLE 4. Tissue Distribution of ^{131}I-IOPA Loaded Estrone Poly(benzyl L-glutamate) Conjugate Microspheres after I.V. Injection Into Rat (n=3) [1,2].

Organ	1 hr mean (s.d.)	3 hr mean (s.d.)	6 hr mean(s.d)	24 hr mean(s.d.)
blood	1.01(0.12)	0.72(0.08)	0.46(0.01)	0.09(0.03)
lung	1.05(0.15)	0.53(0.03)	0.34(0.02)	0.07(0.03)
liver	1.34(0.18)	0.75(0.09)	0.56(0.03)	0.17(0.02)
kidney	0.52(0.01)	0.62(0.05)	0.26(0.01)	0.06(0.02)
uterus	0.70(0.12)	0.62(0.01)	0.39(0.05)	0.06(0.01)
muscle	0.20(0.01)	0.12(0.01)	0.09(0.01)	0.01(0.01)
fat	0.37(0.01)	0.20(0.02)	0.14(0.01)	0.03(0.01)

[1] IOPA = ethyl iopanoic acid.

[2] Data shown represents percent of injected dose per gram tissue.

TABLE 5. Tissue Distribution of ^{131}I-IOPA Loaded Poly(benzyl L-glutamate) Conjugate Microspheres after Intravenous Injection Into Rat (n=3) [1,2].

Organ	1 hr mean(s.d.)	3 hr mean(s.d.)	6 hr mean(s.d)	24 hr mean(s.d.)
blood	1.53(0.71)	1.46(0.21)	1.30(0.26)	0.29(0.16)
lung	1.86(0.40)	1.06(0.16)	1.02(0.19)	0.28(0.03)
liver	1.80(0.78)	1.20(0.15)	1.16(0.14)	0.54(0.16)
kidney	0.74(0.28)	0.58(0.09)	0.58(0.05)	0.24(0.08)
uterus	0.85(0.29)	0.76(0.02)	0.73(0.10)	0.14(0.01)
muscle	0.32(0.16)	0.24(0.02)	0.21(0.03)	0.05(0.01)
fat	0.83(0.29)	0.58(0.23)	0.36(0.11)	0.07(0.04)

[1] IOPA = ethyl iopanoic acid.

[2] Data shown represent percentage of injected dose per gram tissue.

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Relative Tissue Uptake of Modified Microcapsules

Table 6 shows the distribution of ^{131}I-labeled
ethyliopanoate in rats in terms of uterus to muscle
5   ratio. After 3 hr, the targeting of the estrone-
conjugated labeled microcapsules was significantly
greater than targeting by the labeled microcapsules or by
labeled ethyliopanoate.

TABLE 6. Distribution of ^{131}I-Labeled Ethyliopanoate in Rats.

UTERUS TO MUSCLE RATIO

Time (hrs)	1	3	6	24
IOPA[1]	2.92 ± 0.464	3.60 ± 0.346	3.47 ± 0.122	n.d.[3]
PBLG[1]	2.84 ± 0.447	3.23 ± 0.300	3.48 ± 0.369	2.76 ± 0.214
PE[1]	3.50 ± 0.433	5.16 ± 0.592[2]	4.75 ± 0.354[2]	4.25 ± 1.061[2]

[1] IOPA: ethyliopanoate, PBLG: polybenzylglutamate microcapsules loaded with IOPA, PE: microcapsules of estrone and PELG conjugate. Each rate received 5 uCi of radiotracer in saline (0.25 ml).

[2] Significant difference (p<0.05) between PE and the corresponding groups by student T-test.

[3] n.d.: not detectable.

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EXAMPLE 11

Preparation of PEG-IOPA Conjugate

5   Novel hydrophilic microcapsules may be prepared
utilizing hydrophilic polymers for formation of
microcapsules. In the following Example, polyethylene
glycol (PEG) is covalenthy attached to a labeling agent,
iopanoic acid. It is contemplated that this material may
10   be radily formulated into microcapsules according to
Example 1.

      Into 6 mL methylene chloride solution containing
1.45 g poly(ethylene glycol) (PEG, MW 1450, 1.0 mmol) was
15   added 1.43 g iopanoic acid (IOPA, 2.5 mmol), 454 mg
dicyclohexylcarbodiimide (DCC, 2.2 mmol) and 24 mg
dimethylaminopyride (0.24 mmol). The reaction mixture
was stirred overnight at room temperature. After
filtration to remove dicyclohexylurea (DCU) precipitatek
20   the solution was evaporated to dryness. The residual was
then washed with dry ether three times to yield a
hygroscopic solid. Yield 1.15 g(45%). Iodine content
20-30% (w/w). Using the microcapsule preparation
according to Example 1 the product is then formulated as
25   particles (<5μm) which .re suitable for iv injection.

      In vivo biodistribution of ^{131}I-IOPA attached to
polyethylene glycol (PEG) is shown in Tables 7 and 8.
The ratio of liver to blood uptake of ^{131}I-IOPA is altered
30   by the presence of PEG which is hydrophilic. The changes
in liver/blood uptake ratio conferred by conjugation of
^{131}I-IOPA to PEG are illustrated in Figure 23.

Tabl 7. Organ Distribution of ^{131}I PEG-IOPA Solution After Intravenous Injection to Rats (n=3)

Organ	10 min	1 hr	3 hr	6 hr
blood	1.67 (0.05)	1.62 (0.19)	0.54 (0.13)	0.39 (0.18)
lung	0.65 (0.08)	0.38 (0.14)	0.06 (0.02)	<0.01
spleen	0.64 (0.04)	0.24 (0.04)	0.03 (0.01)	<0.01
liver	2.74 (0.50)	1.28 (0.12)	1.00 (0.02)	0.55 (0.09)
kidney	1.78 (0.13)	0.83 (0.09)	0.49 (0.07)	0.27 (0.06)
muscle	<0.01			
liver/blood	1.64 (0.34)	0.80 (0.10)	1.92 (0.47)	1.53 (0.43)

5

10

Tabl 8. Organ Distribution of ^{131}I PEG-IOPA Suspension After Intravenous Injection to Rats (n=3)

Organ	20 min	1 hr	3 hr	6 hr
blood	3.18 (0.38)	2.01 (0.06)	2.15 (0.48)	1.36 (0.06)
lung	1.73 (0.14)	1.01 (0.11)	0.93 (0.25)	0.98 (0.13)
liver	2.72 (0.95)	1.52 (0.05)	1.57 (0.33)	1.29 (0.08)
kidney	1.72 (0.07)	0.99 (0.04)	1.09 (0.18)	0.88 (0.11)
muscle	0.89 (0.14)	0.44 (0.08)	0.40 (0.06)	0.26 (0.02)
liver/blood	0.85 (0.24)	0.73 (0.03)	0.76 (0.02)	0.95 (0.10)

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The present invention has been described in terms of
particular embodiments found by the inventors to comprise
preferred modes of practice of the invention. It will be
appreciated by those of skill in the art that in light of
5   the present disclosure numerous modifications and changes
can be made in the particular embodiments exemplified
without departing from the intended scope of the
invention. For example, amino acid modified
microcapsules could be attached to specific targeting
10   agents without affecting the intended nature and practice
of the invention. All such modifications are intended to
be included within the scope of the claims.

                    ****

15

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by reference to the extent they supplement, explain,
20   provide a background for or teach methodology, techniques
and/or compositions employed herein.

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15   Although the present invention has been described in
some detail by way of illustration and example for
purposes of clarity and understanding, it will be obvious
that certain changes and modifications may be practiced
within the scope of the claims.

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CLAIMS

1.   Hydrophilic polyglutamate microcapsules having a
diameter of about 1-250 μm useful for *in vivo*

5   biodistribution.

2. . The hydrophilic polyglutamate microcapsules of claim
1 characterized as having surface-attached alkyl hydroxyl

10   groups.

3.   The hydrophilic polyglutamate microcapsules of claim
2 wherein the alkyl moiety is 1-5 carbon atoms.

15

4.   The hydrophilic polyglutamate of claim 1 wherein the
attached hydroxyl group is propylhydroxyl.

20

5.   Hydrophilic PEG microcapsules having a diameter of
about 1-5μm wherein the PEG is corulently bound to
iopanoic acid by a covalent amide bond and wherein said
microcapsules are suitable for intravenous injection.

25

6.   A pharmaceutical composition comprising an amount of
poly(hydroxypropyl)-L-glutamate microcapsules combined
with a therapeutic or diagnositic agent in a

30   pharmaceutically acceptable diluent suitable for
intravascular administration.

7.   The pharmaceutical composition of claim 6 wherein

35   the poly(hydroxylpropyl)-L-glutamate microcapsules have
an average diameter of about 1 to about 250 μm.

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8.   The pharmaceutical composition of claim 6 wherein the diagnostic agent is an imaging agent.

5   9.   The pharmaceutical composition of claim 8 wherein the imaging agent is metrizoate, iothalamate, iohexol, ioxaglate, ioxalen or Gd-DPTA.

10   10.   The pharmaceutical composition of claim 8 wherein the therapeutic agent is cisplatin, 5-fluorouracil or Tamoxifen.

15   11.   A method of selective *in vivo* delivery of a pharmaceutical agent to a tissue comprising administering to a mammal an effective amount of a pharmaceutically acceptable preparation of the hydrophilic microcapsules according to claim 1 wherein said microcapsules contain 20   the pharmaceutical agent.

12.   The method of claim 11 wherein the administering is by intravascular injection.

25

13.   The method of claim 11 wherein the pharmaceutical agent is covalently attached to the microcapsule.

30

14.   The method of claim 11 wherein the pharmaceutical agent is entrapped in the microcapsule.

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15. The method of claim 11 wherein the pharmaceutical
agent is estrone attached to poly hydroxypropyl-L-
glutamate microspheres.

5   16. The method of claim 15 wherein the estrone is
selectively delivered to uterus tissue.

17. A method of preparing hydrophilic microcapsules for
10   in vivo cell targeting, comprising the steps:

        obtaining polybenzyl-L-glutamate microspheres having
            a diameter of about 1 to about 250 μm;

15   modifying the microspheres with an aminoalkyl
                alcohol in the presence of a crosslinker to
                obtain surface modification of said
                microspheres; and

20   collecting the hydrophilic microcapsules.

18. The method of claim 17 wherein microspheres of about
    1 μm diameter are obtained by combining an aqueous
25 solution, a nontoxic emulsifier and the polymer dissolved
    in an organic solvent, vigorously agitating the
    combination for at least five minutes and terminating the
    agitating when an average microcapsule diameter of about
    1 μm diameter is obtained.
30

19. The method of claim 17 wherein microspheres of about
    200-300 μm diameter are obtained by admixing the polymer
    in an organic solvent with a solution of a nontoxic
35 emulsifier, agitating the mixture at a low agitation rate

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and terminating the agitation when presence of an average
microcapsule diameter of about 100 μ is indicated.

5   20.   The method of claim 17 further comprising admixing
tyrosine with the aminoalkyl alcohol in the presence of
the crosslinker.

10   21.   The method of claim 17 wherein the aminoalkyl
alcohol is aminopropyl alcohol.

22.   The method of claim 17 wherein the crosslinker is
15   diaminohexane.

23.   A modified poly-L-glutamate microcapsule having a
diameter of about 1 to about 250 μm with amide linkages
20   to 3-aminopropyl alcohol according to Figure 21A.

24.   A modified poly-L-glutamate microcapsule having a
diameter of about 1 to about 220 μm with amide linkages
25   to 4-methyamino tyrosine according to Figure 21B.

25.   The modified poly-L-glutamate microcapsule of claim
24 wherein the phenolic moiety is iodinated.

30

26.   The modified poly-L-glutamate microcapsule of claim
25 wherein the iodination is with [131]-labeled iodine.
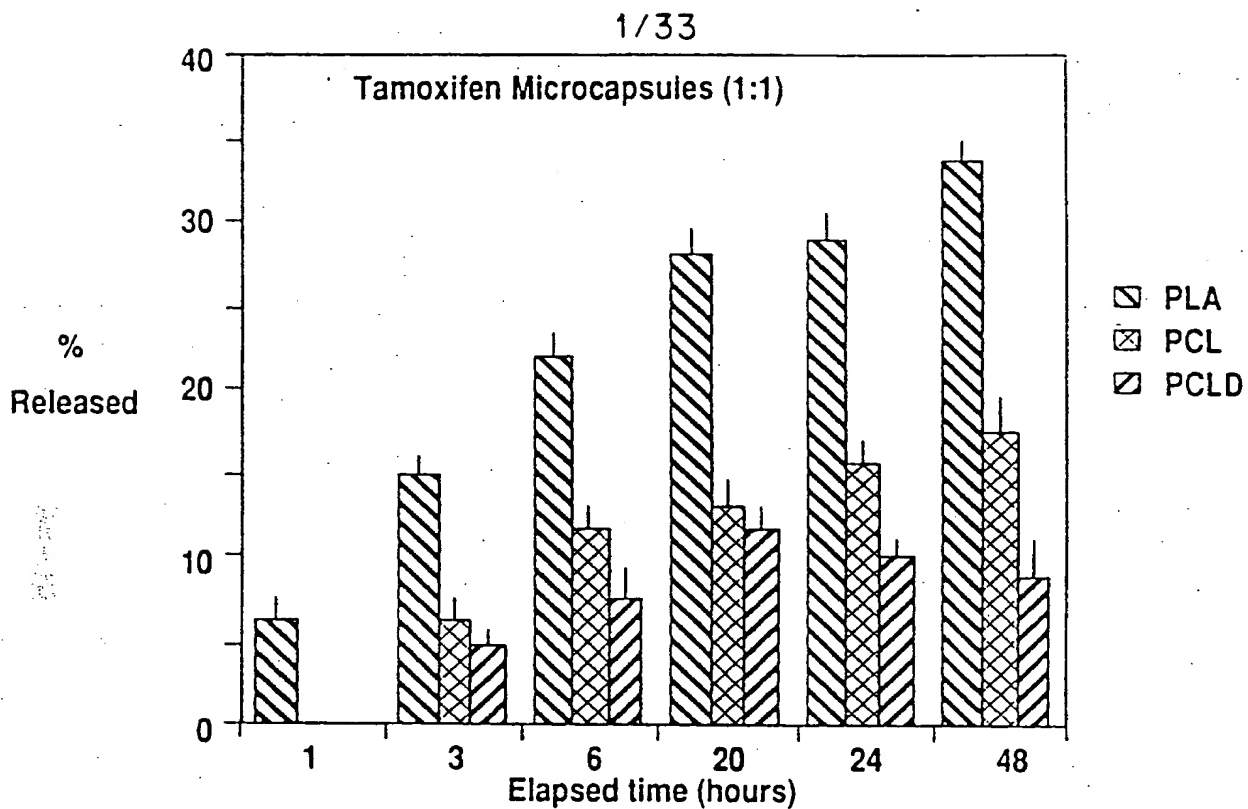
FIG. 1
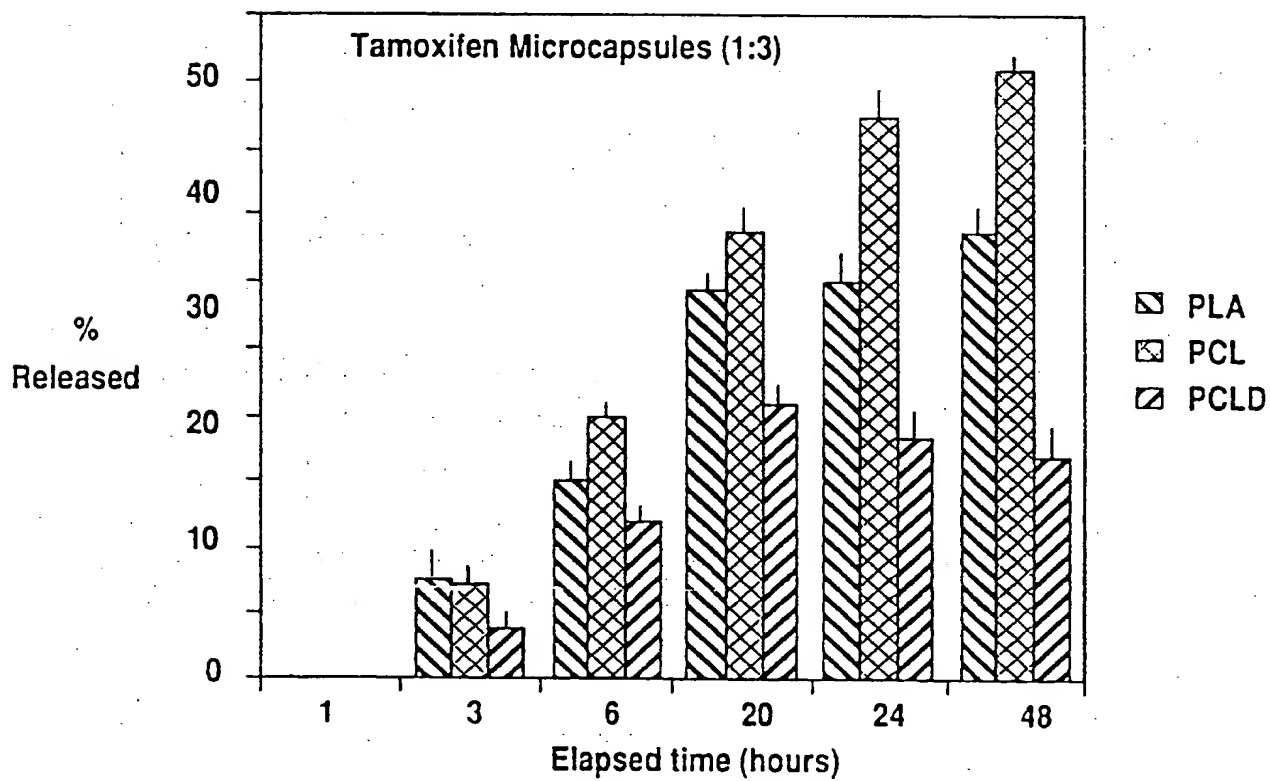
FIG. 2
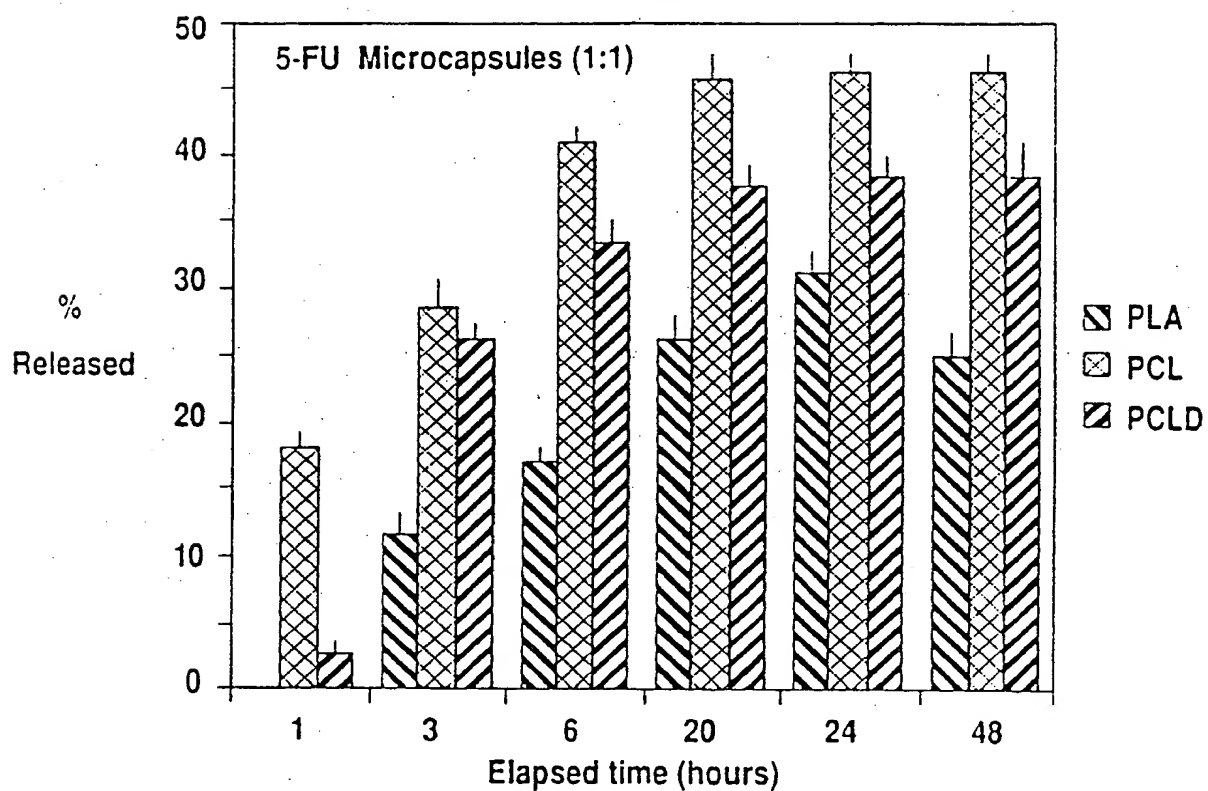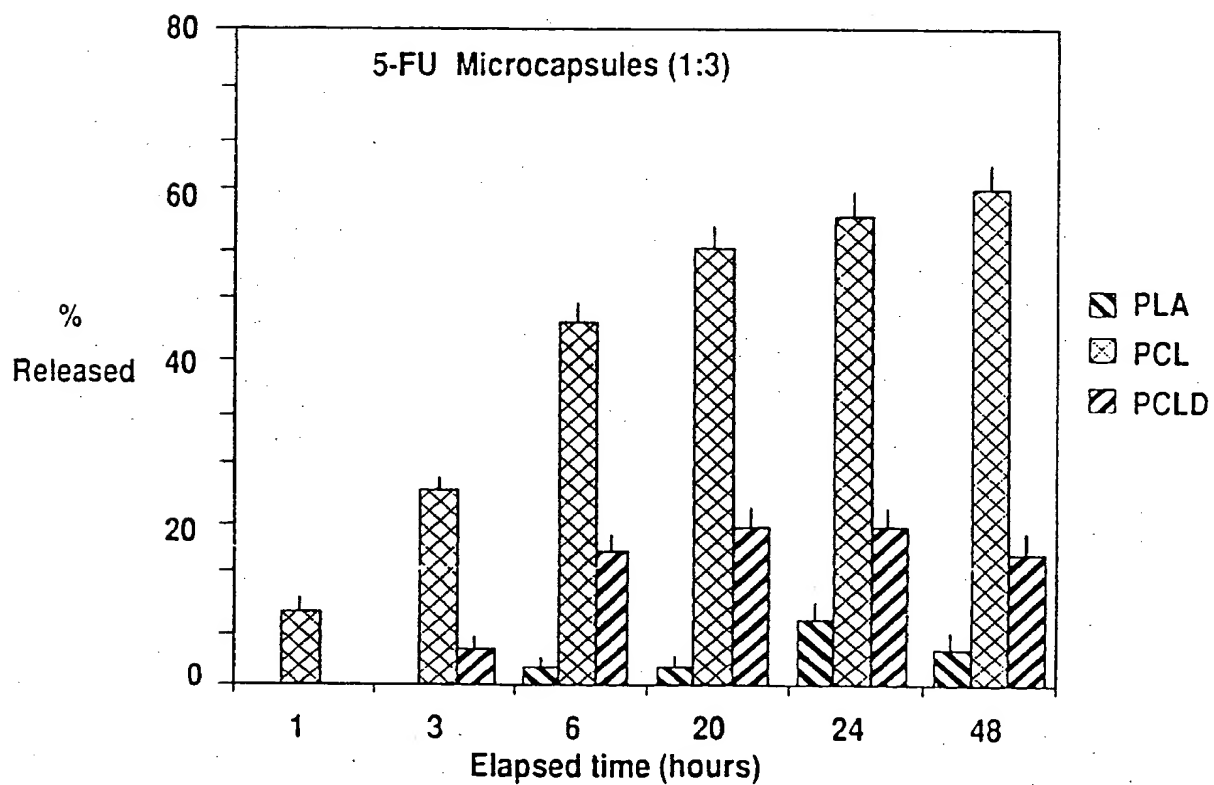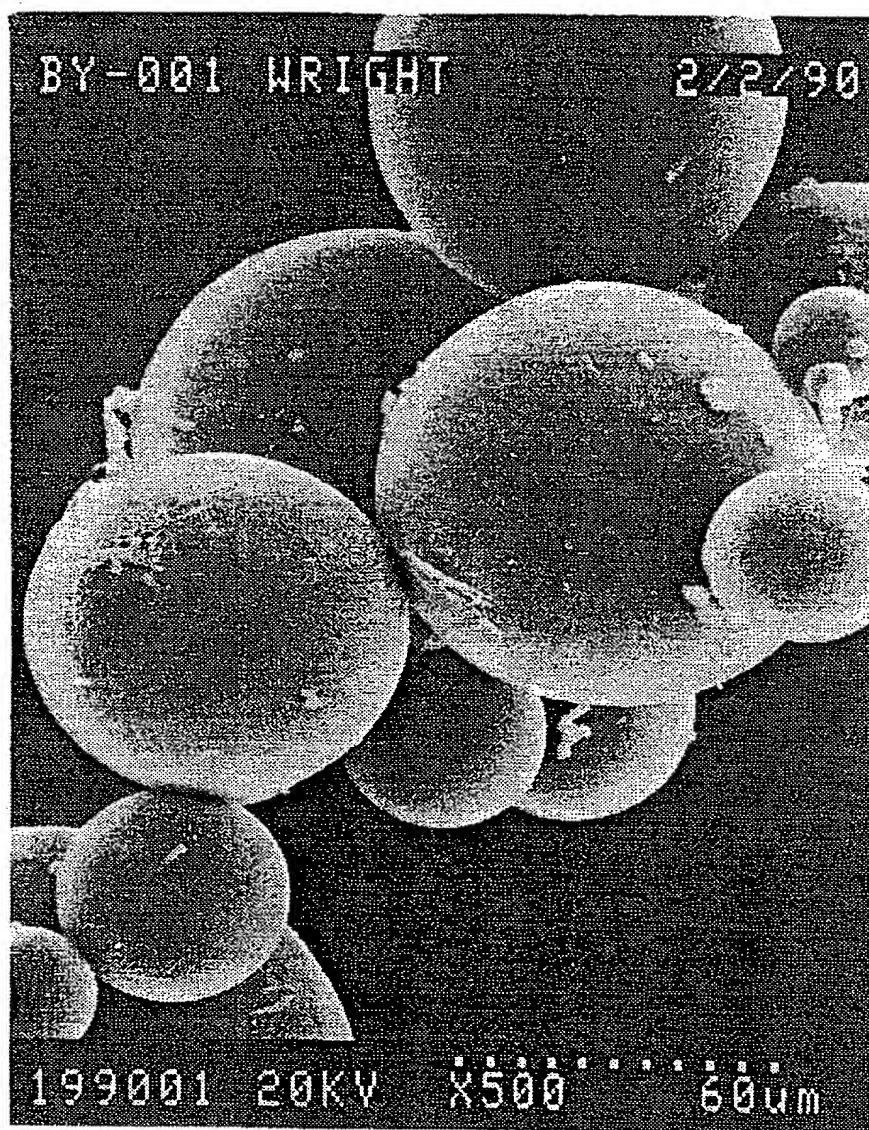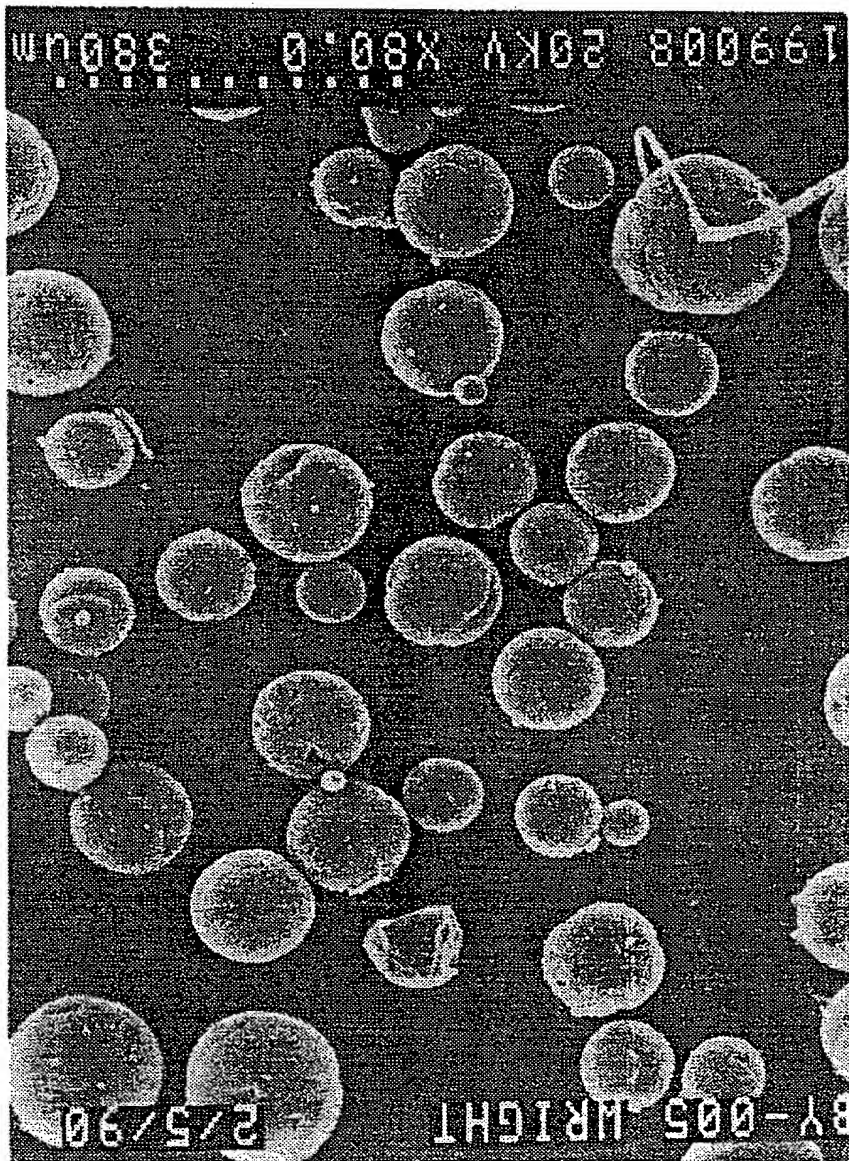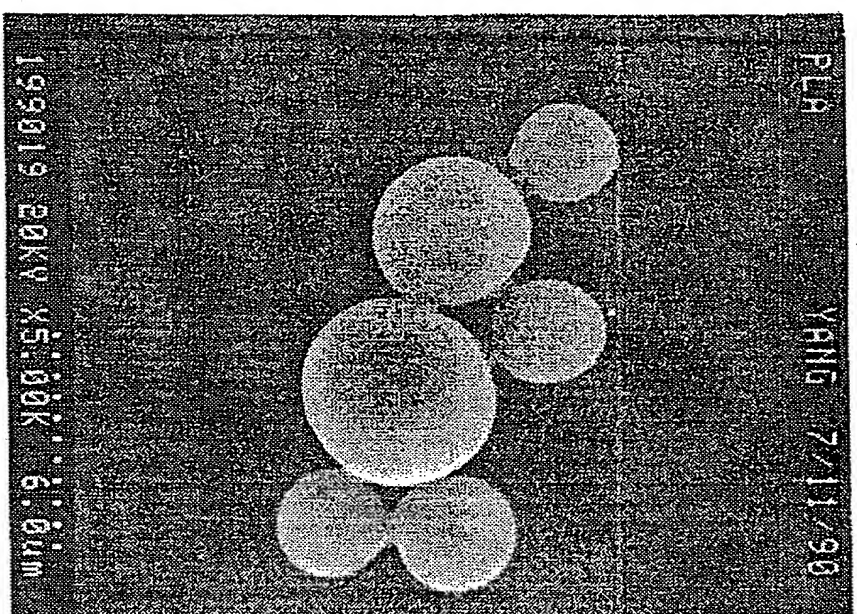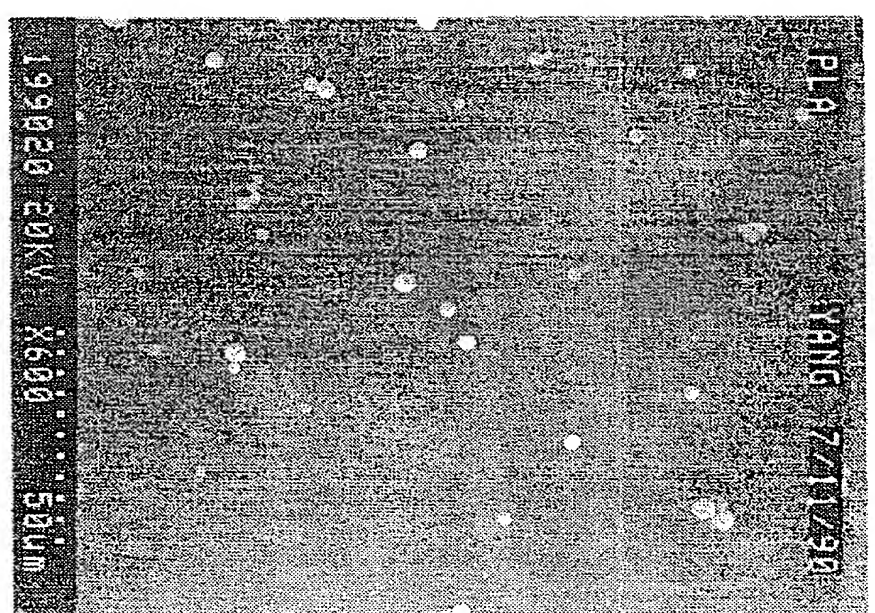
FIG. 3

FIG. 4

BY-001 WRIGHT 2/2/90

199001 20KV X500 60um

# FIG. 5

FIG. 6

FIG. 7A

FIG. 7B

SAMPLE : 0

1000=100%   256 CHANNEL FULL RANGE
                          ACCUMULATION 07/06/90

50%

Edit On WL = 011/4.659E0um   Wu = 037/7.206E0um
        $\Delta L = 465$ $\Sigma = 2527$ $\Delta u = 2$
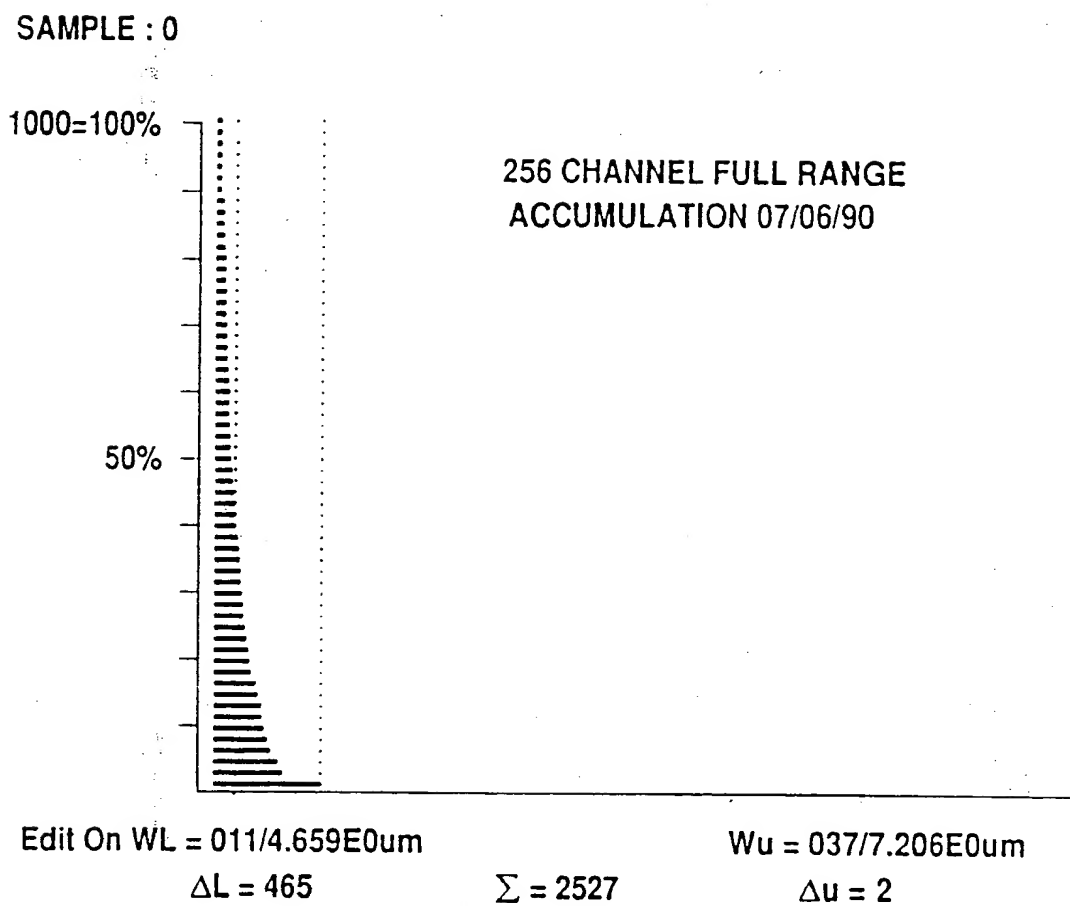
FIG. 8

SAMPLE : 0

1000=100%   256 CHANNEL FULL RANGE
                   ACCUMULATION 07/06/90

50%

Edit On WL = 004/3.119E0um   Wu = 037/7.206E0um
        $\Delta L = 0$ $\Sigma = 6404$ $\Delta u = 4$

FIG. 9
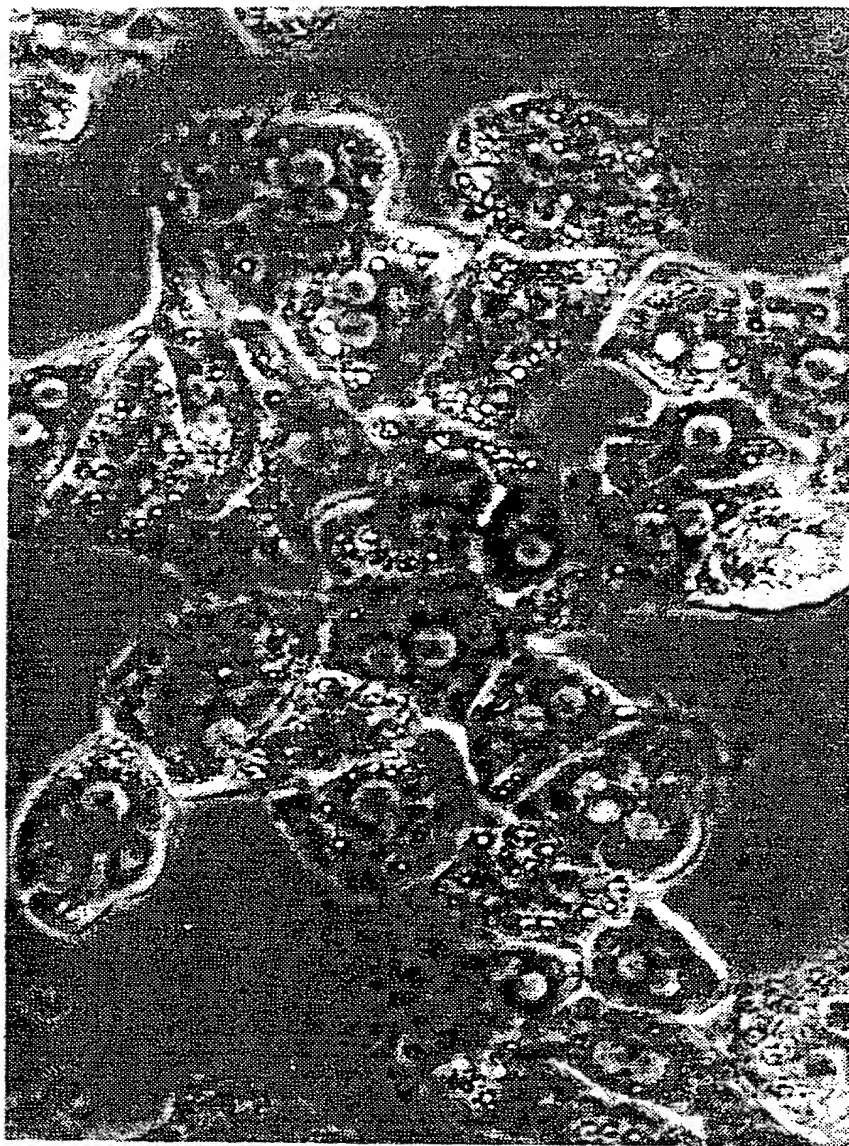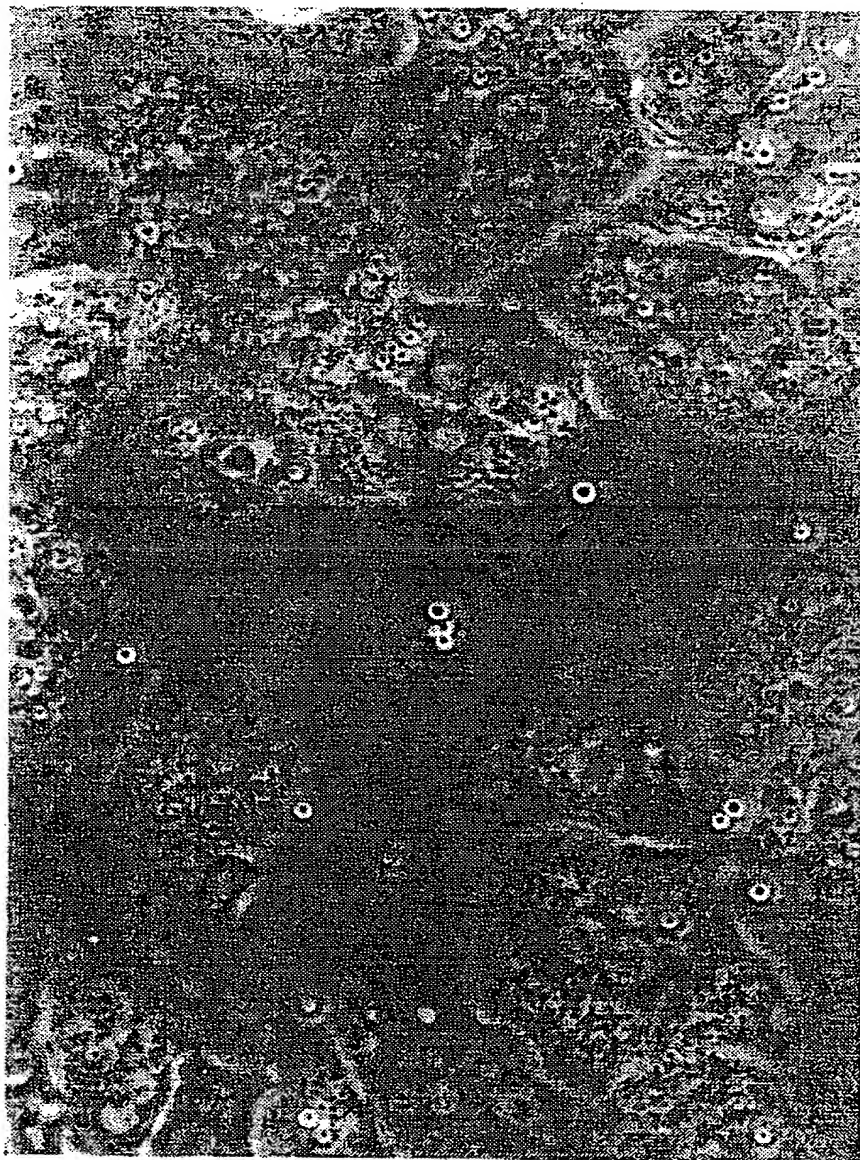
FIG. 10A

FIG. 10B
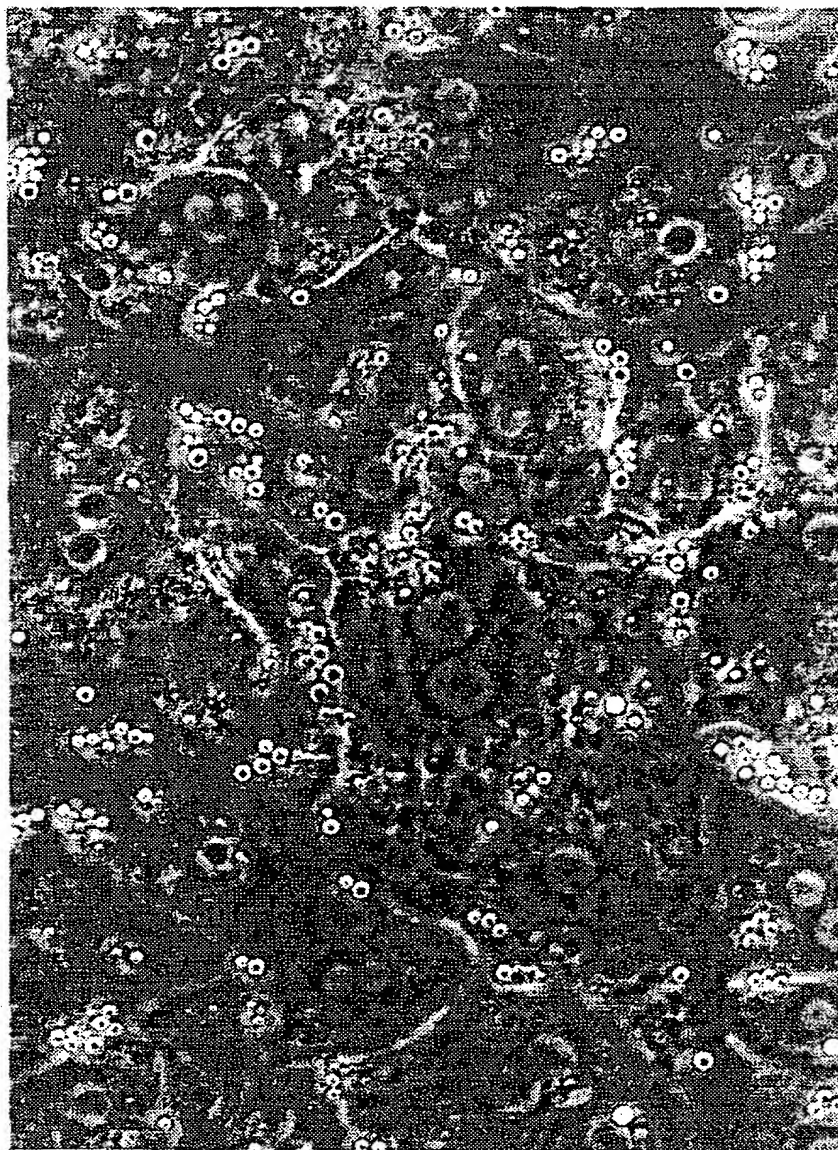
FIG. 10C
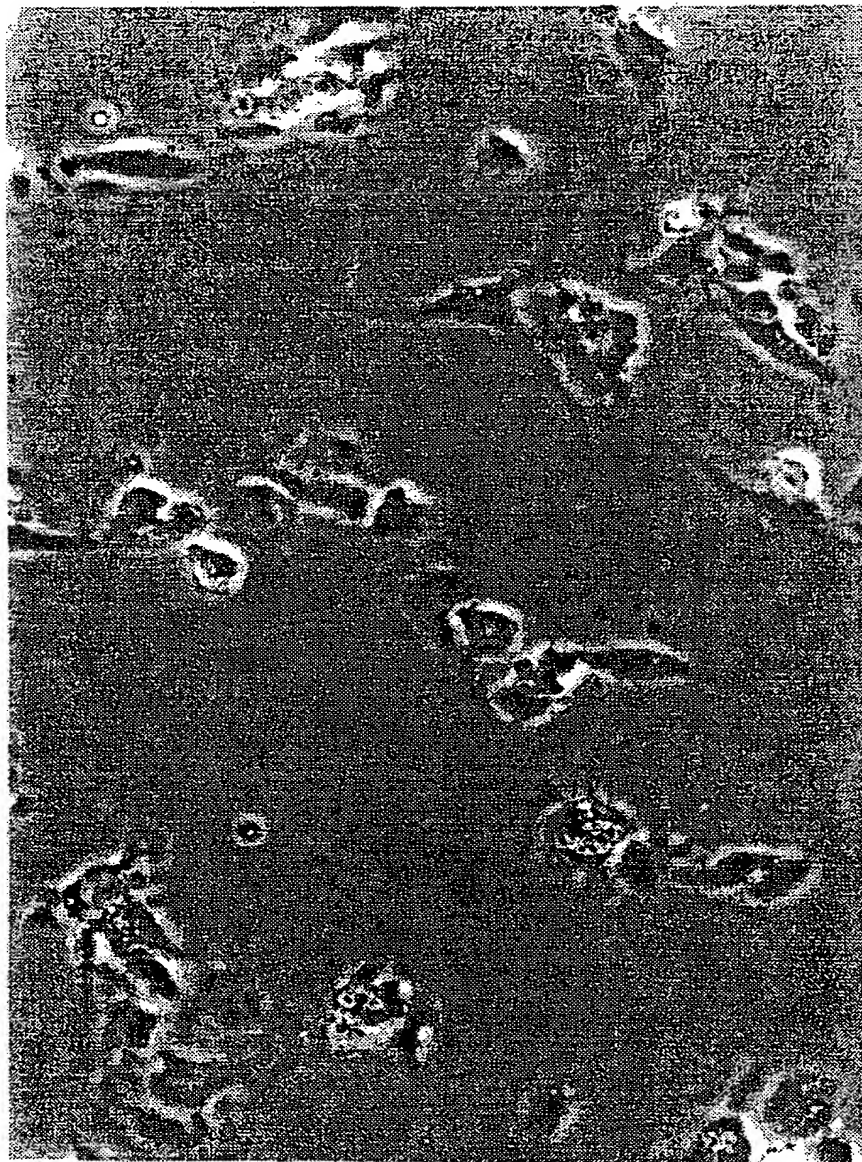
FIG. 11A
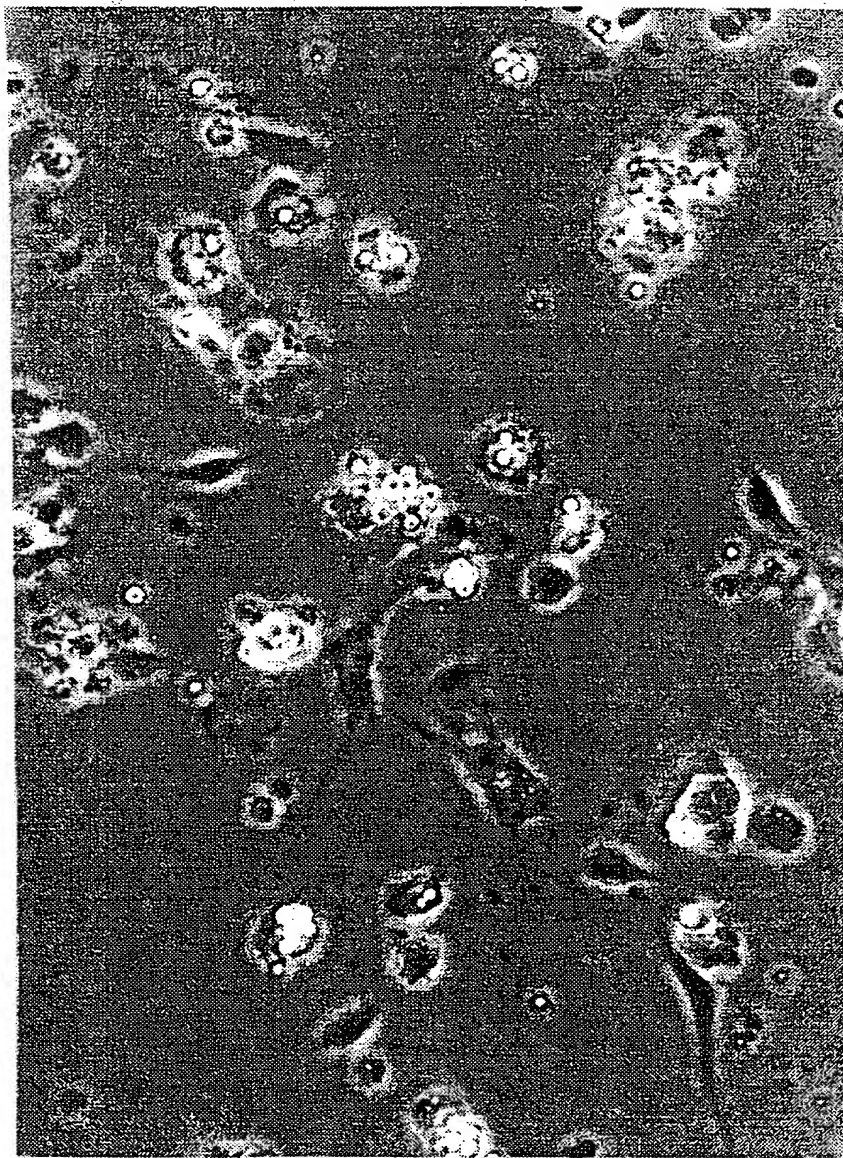
FIG. 11B
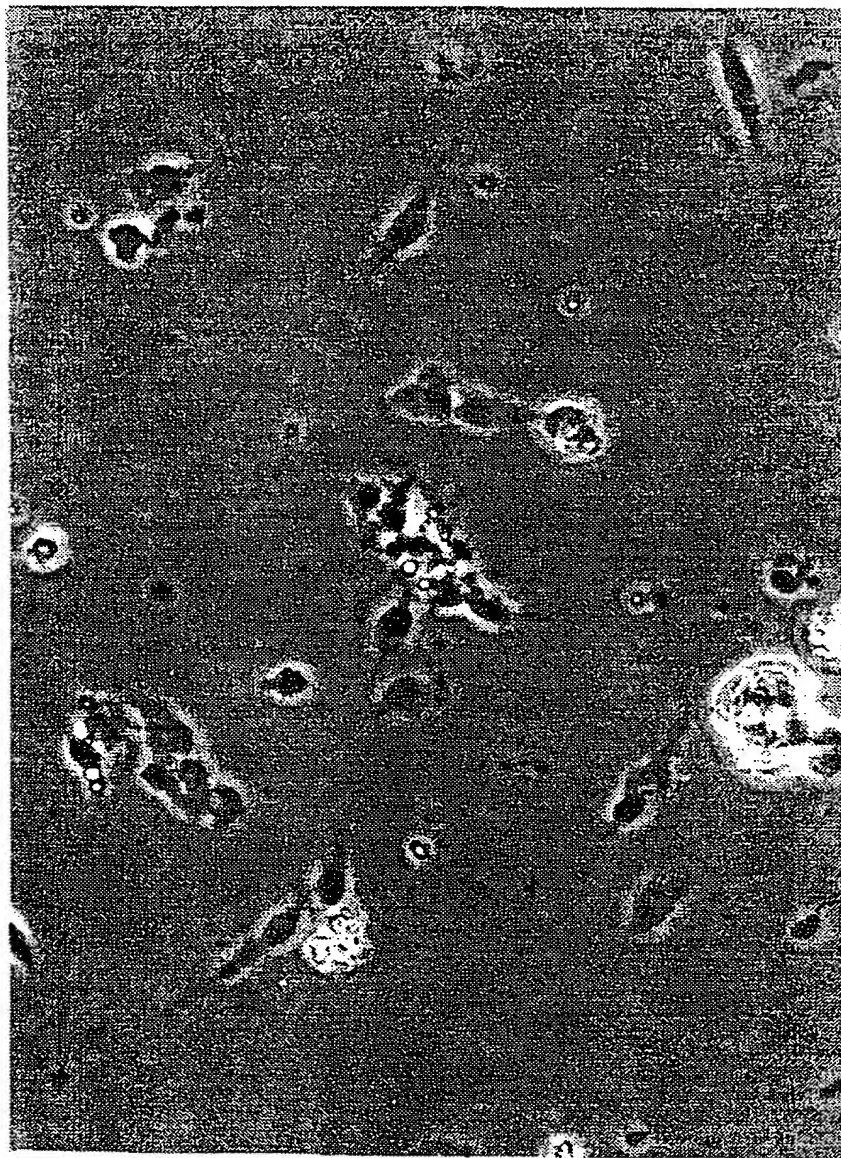
FIG. 11C

FIG. 12G
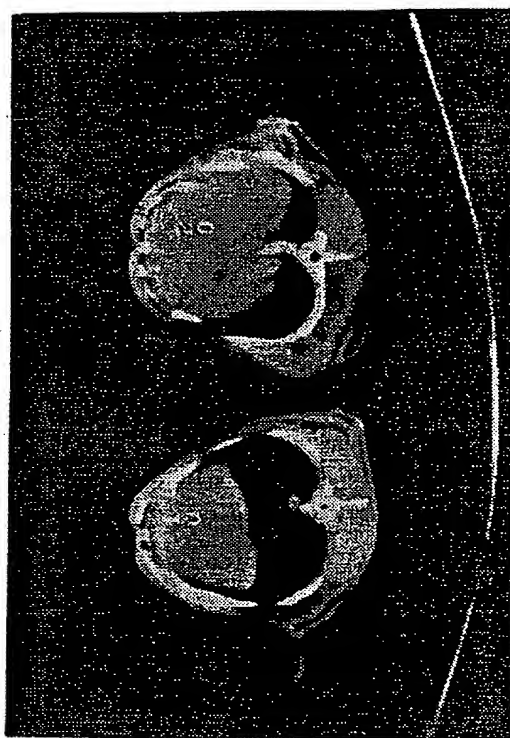
FIG. 12A

FIG. 12H
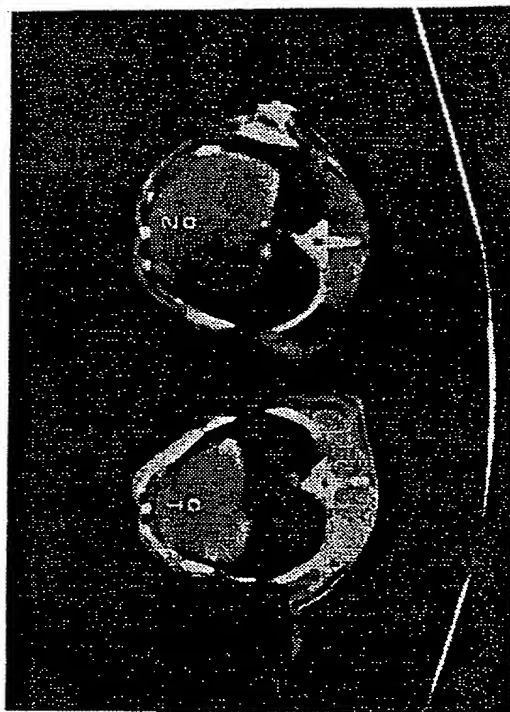
FIG. 12B

FIG. 12I
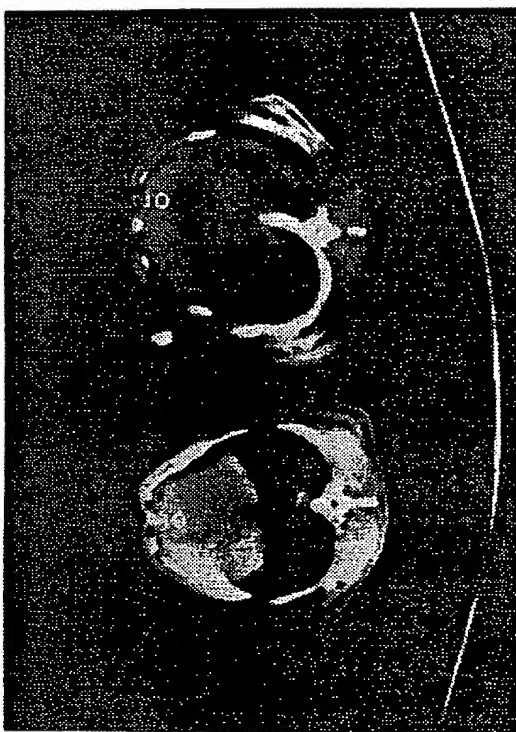
FIG. 12C

FIG. 12J
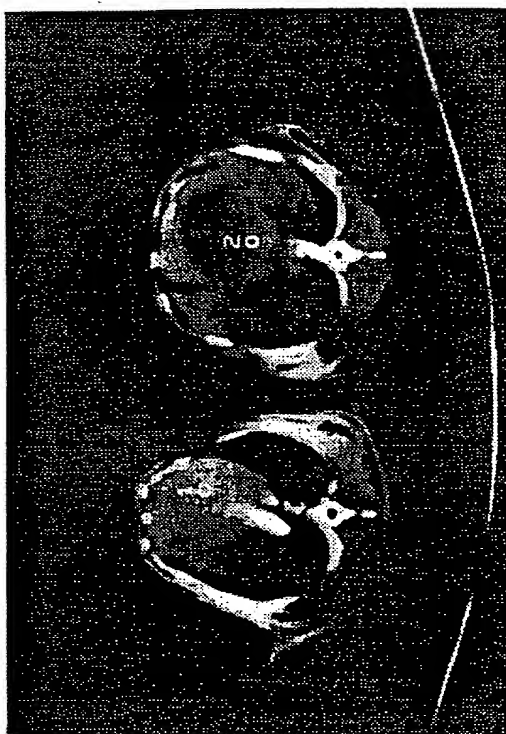
FIG. 12D

FIG. 12K
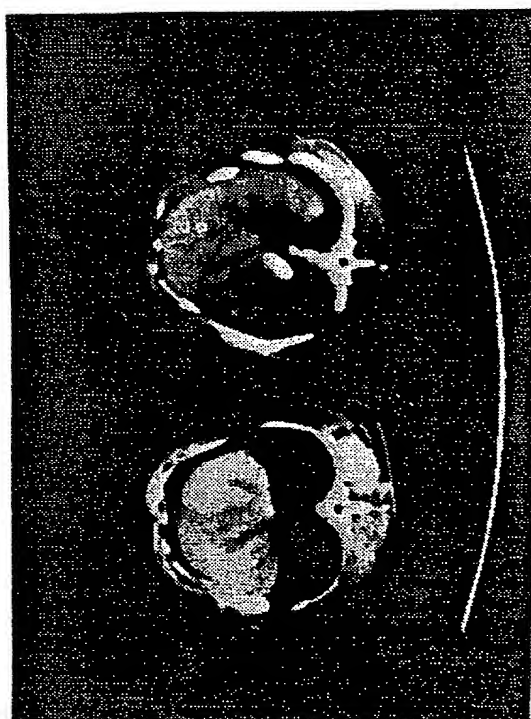
FIG. 12E

FIG. 12L
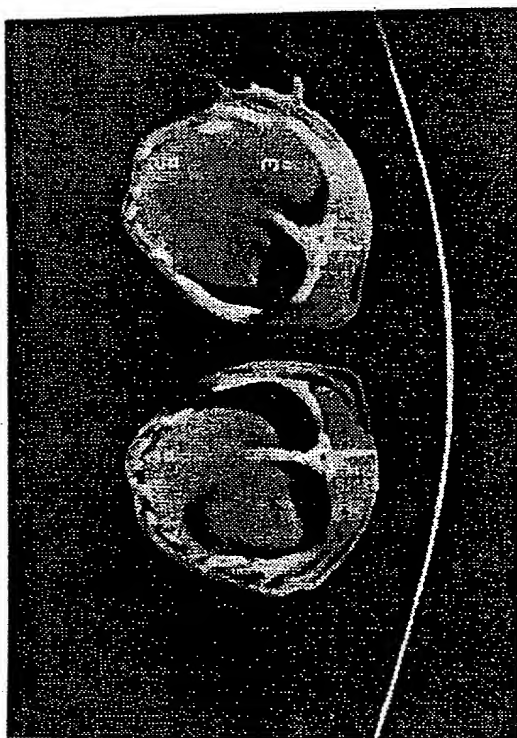
FIG. 12F
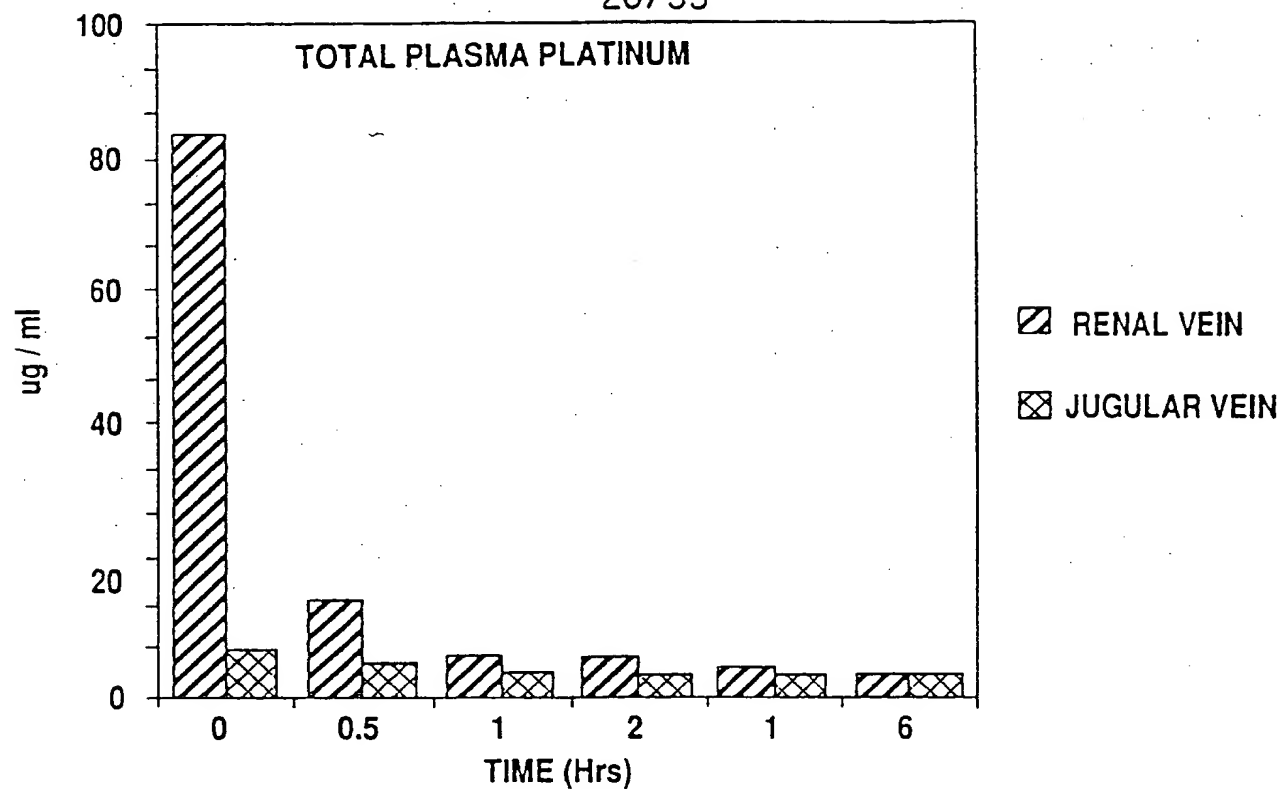
F I G. 13
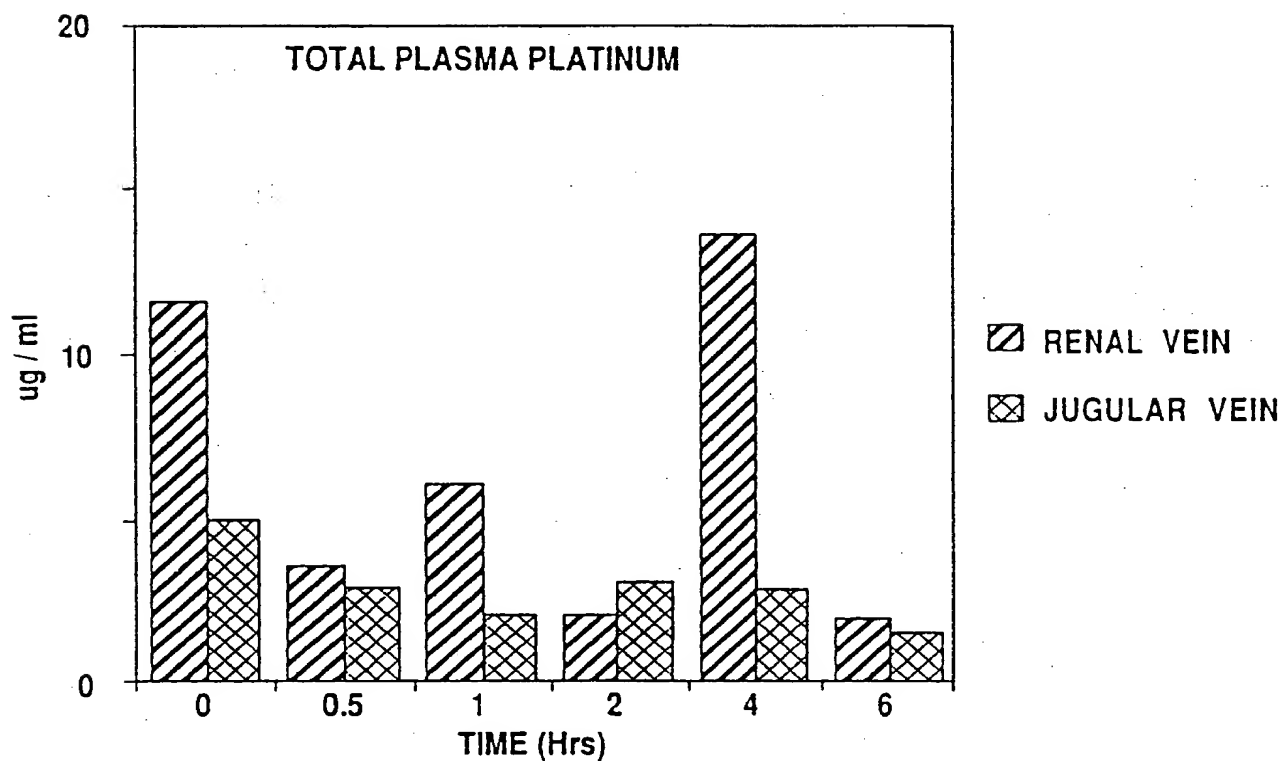
F I G. 14
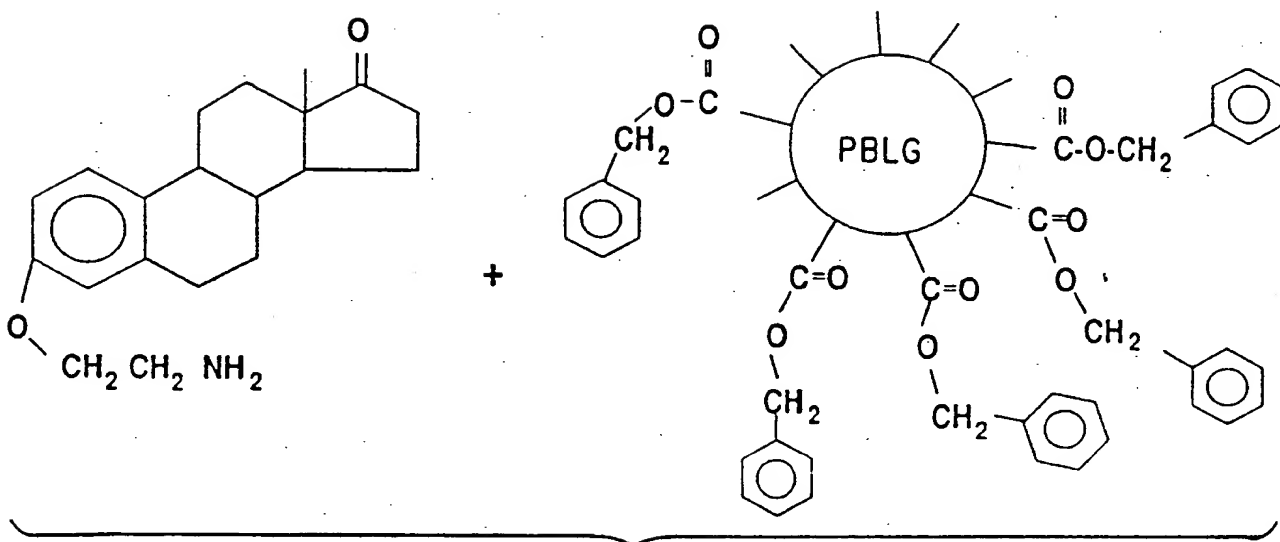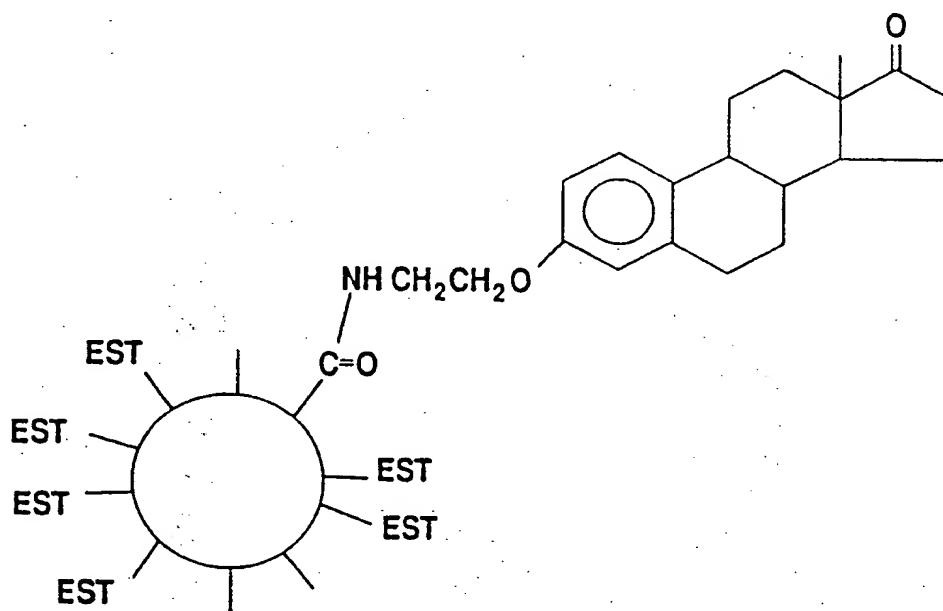
FIG. 15A

FIG. 15B
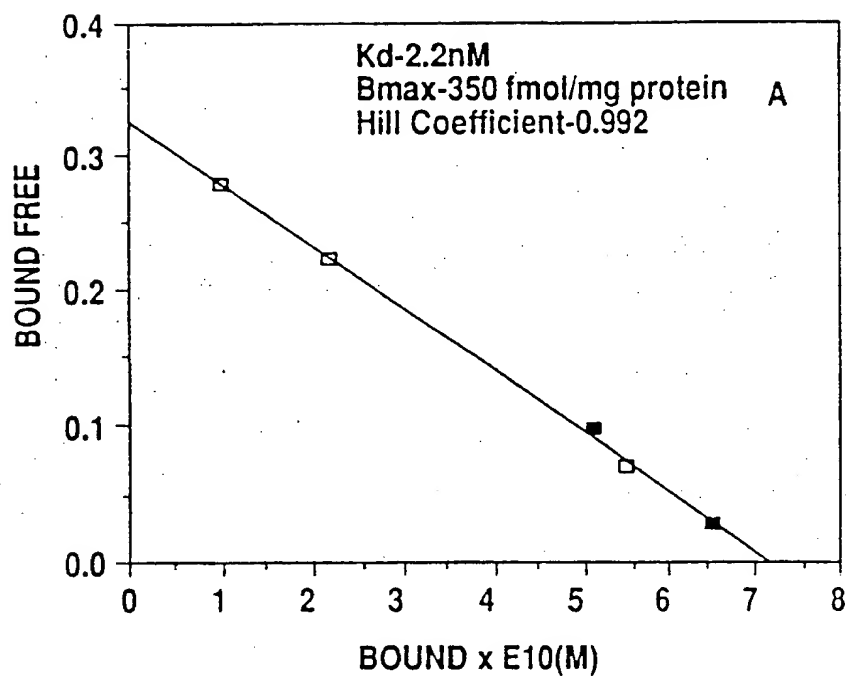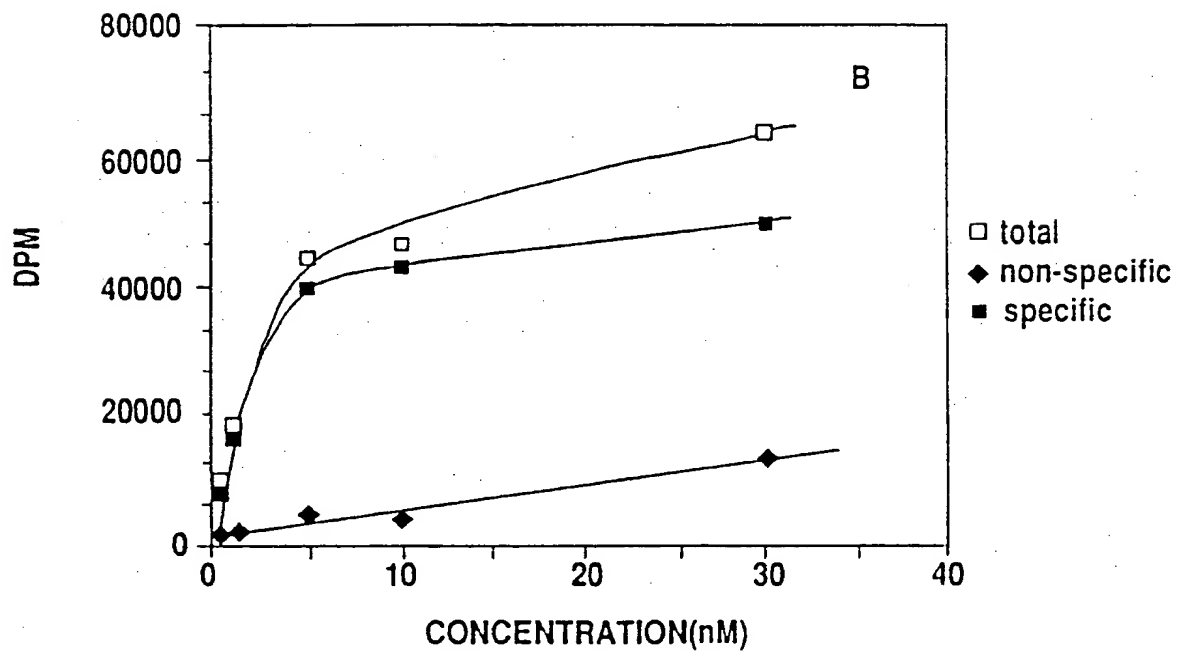
FIG. 16A

FIG. 16B
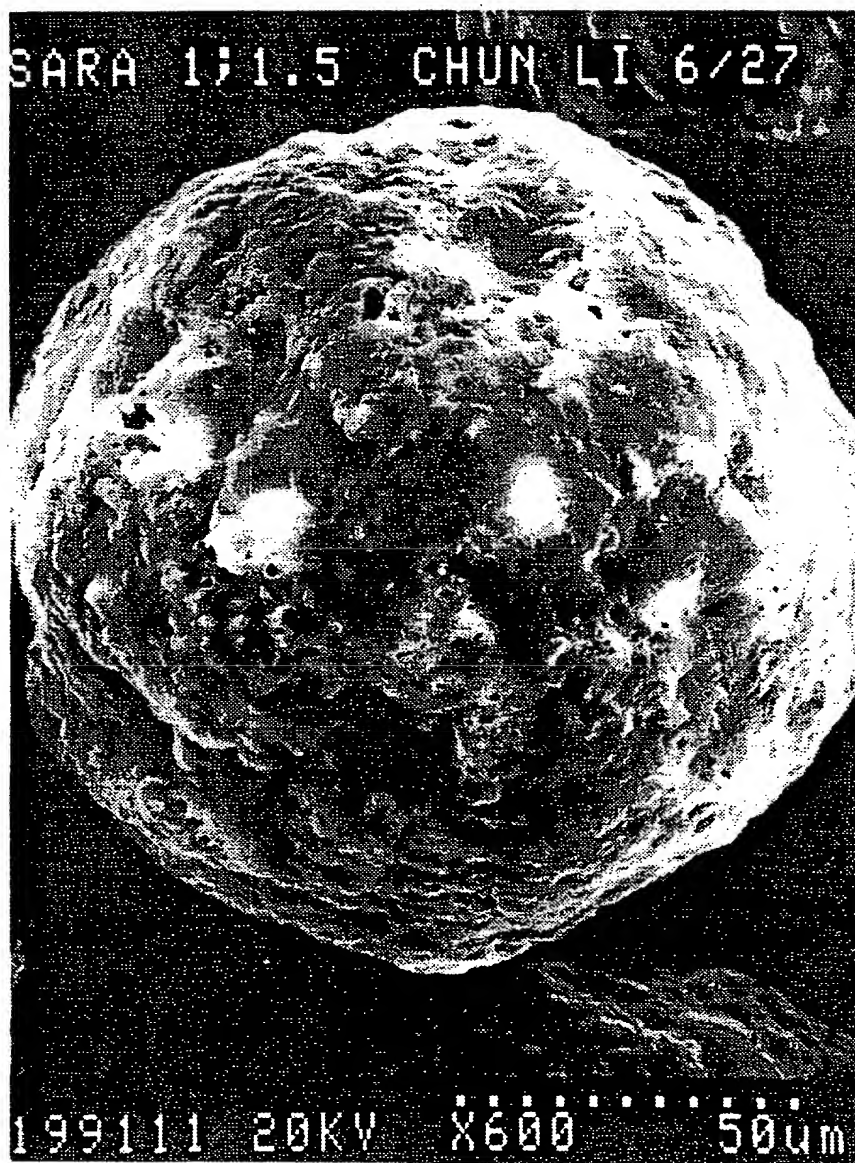
FIG. 17A
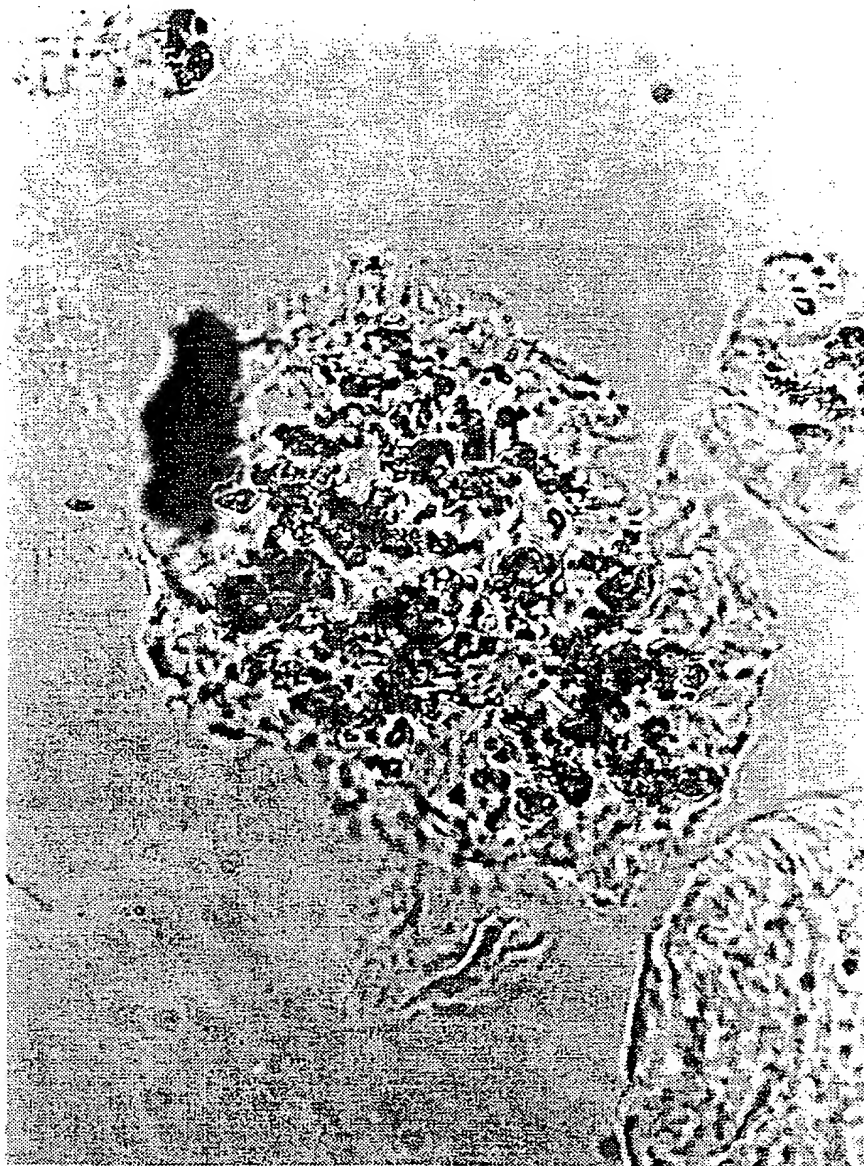
FIG. 17B
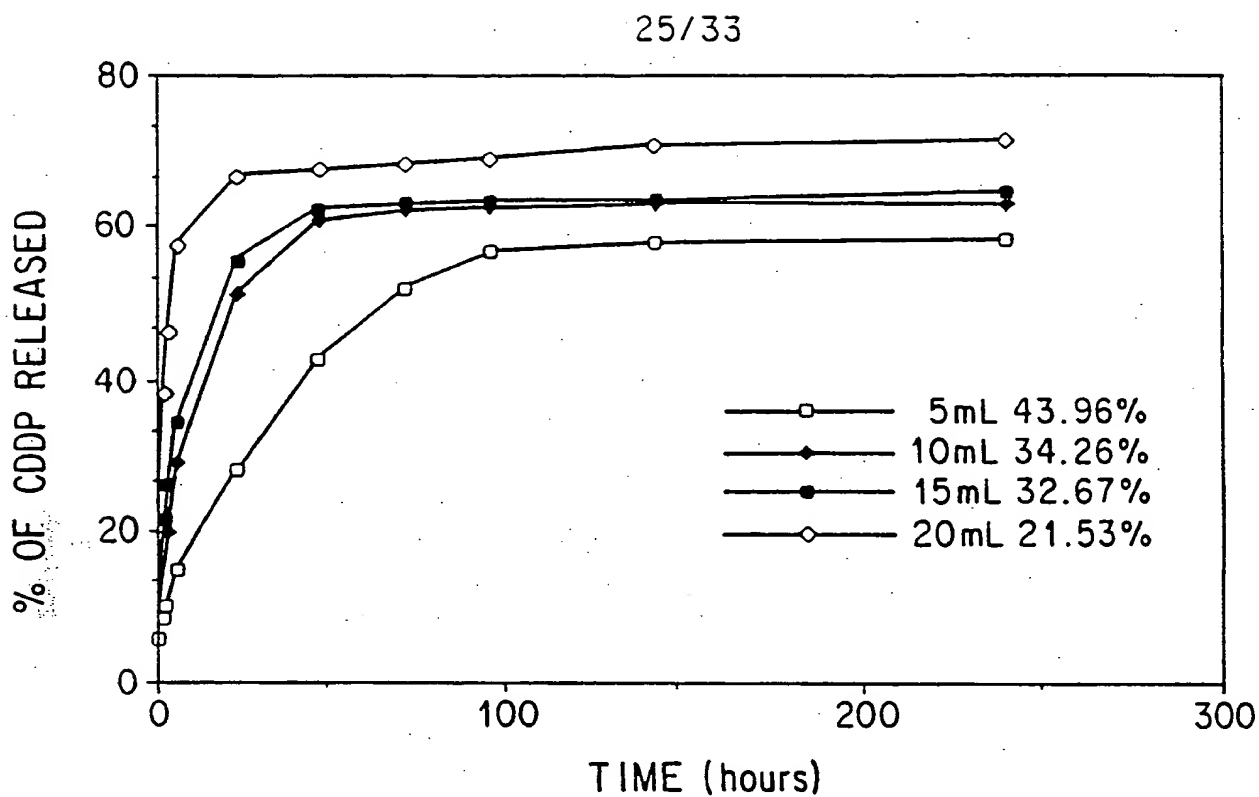
F I G. 18
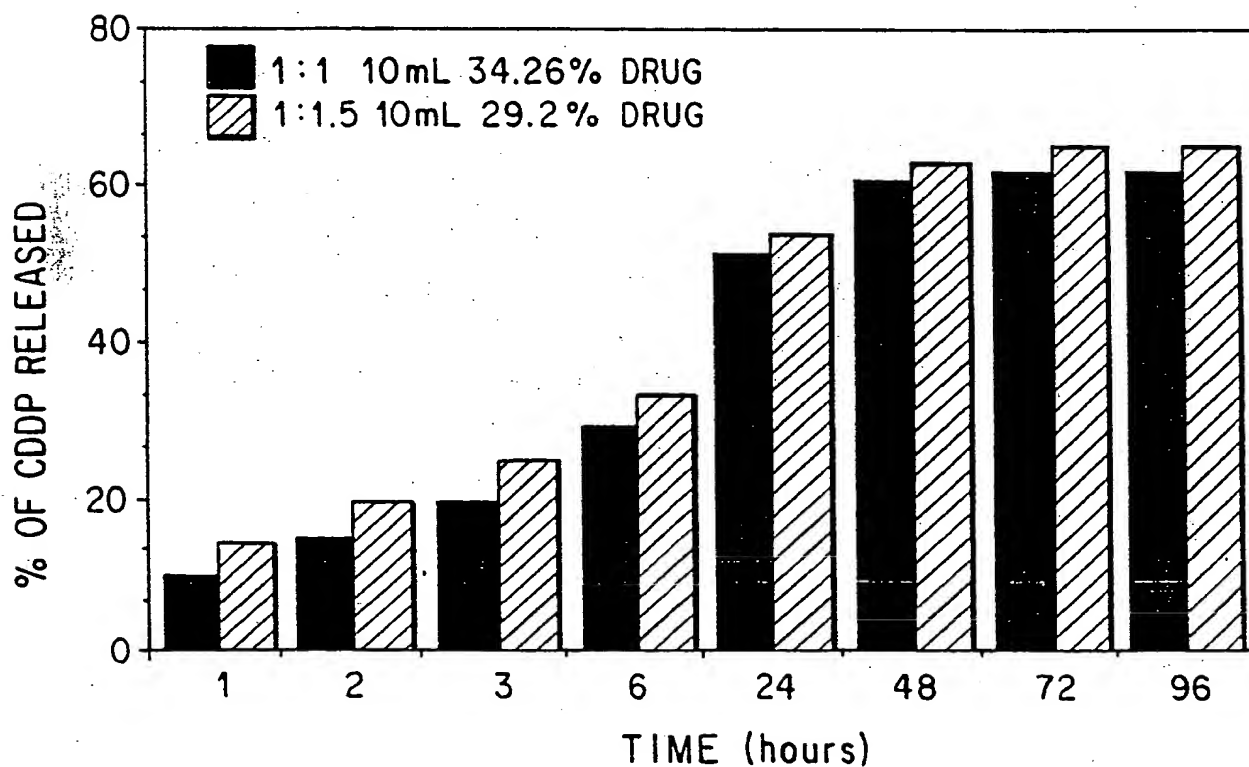
F I G. 19
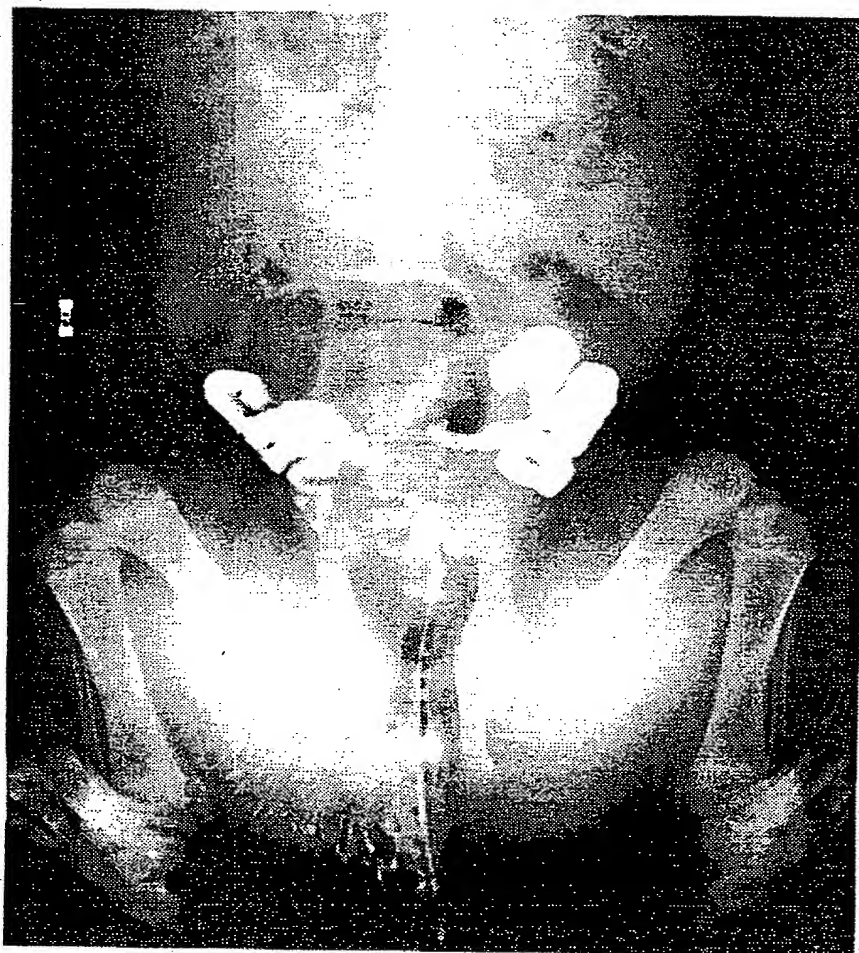
F I G. 20A
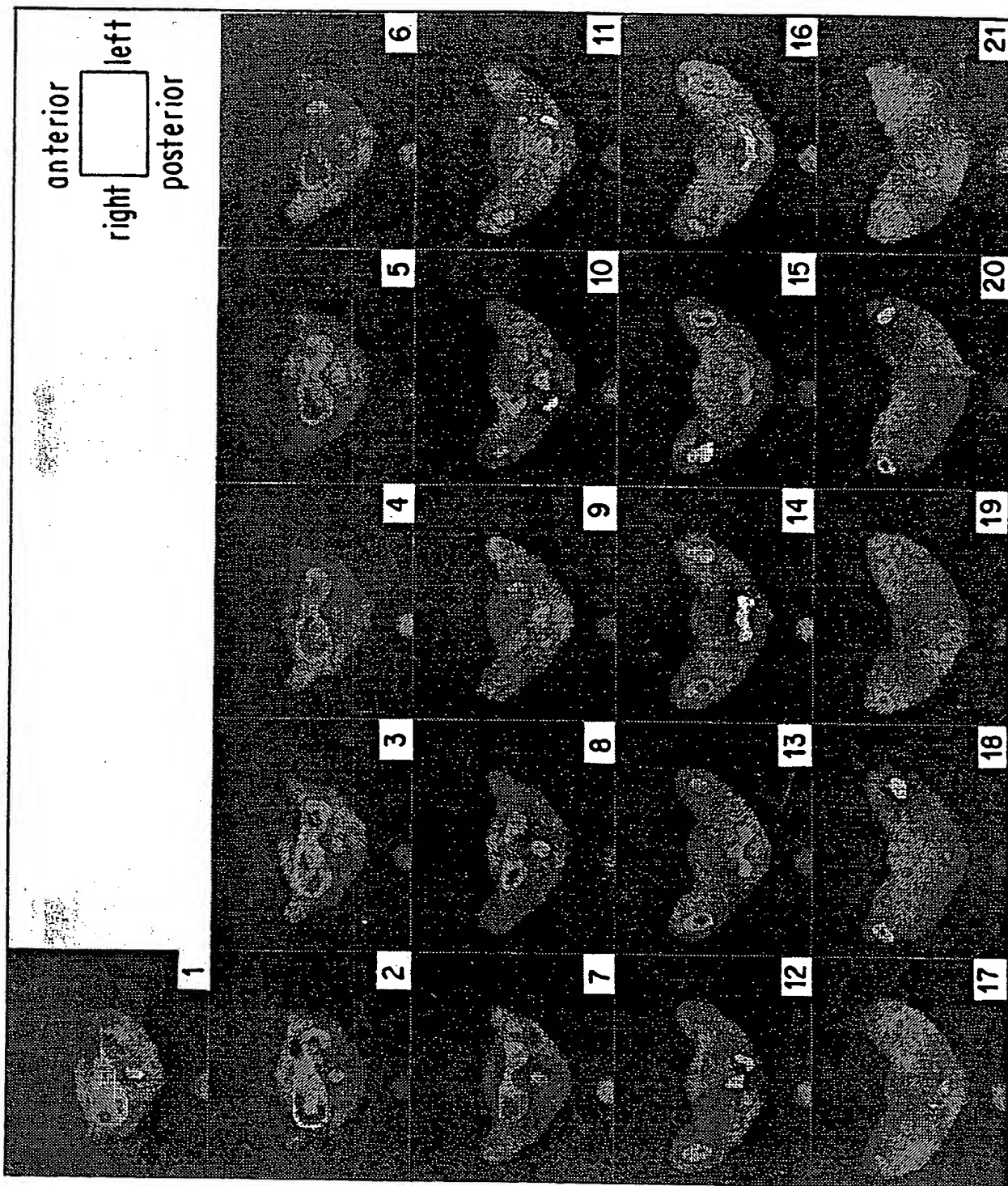
FIG. 20B
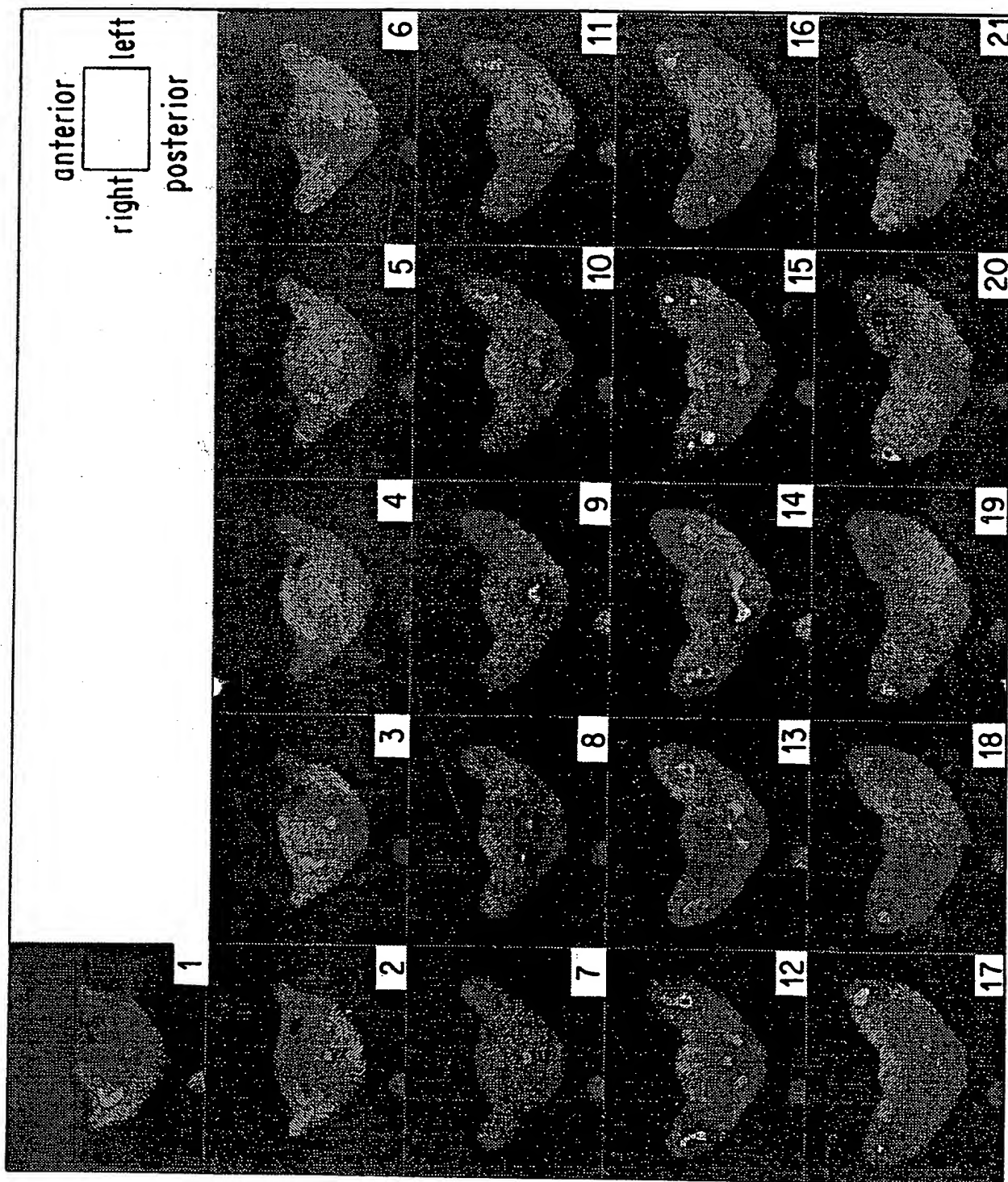
FIG. 20C
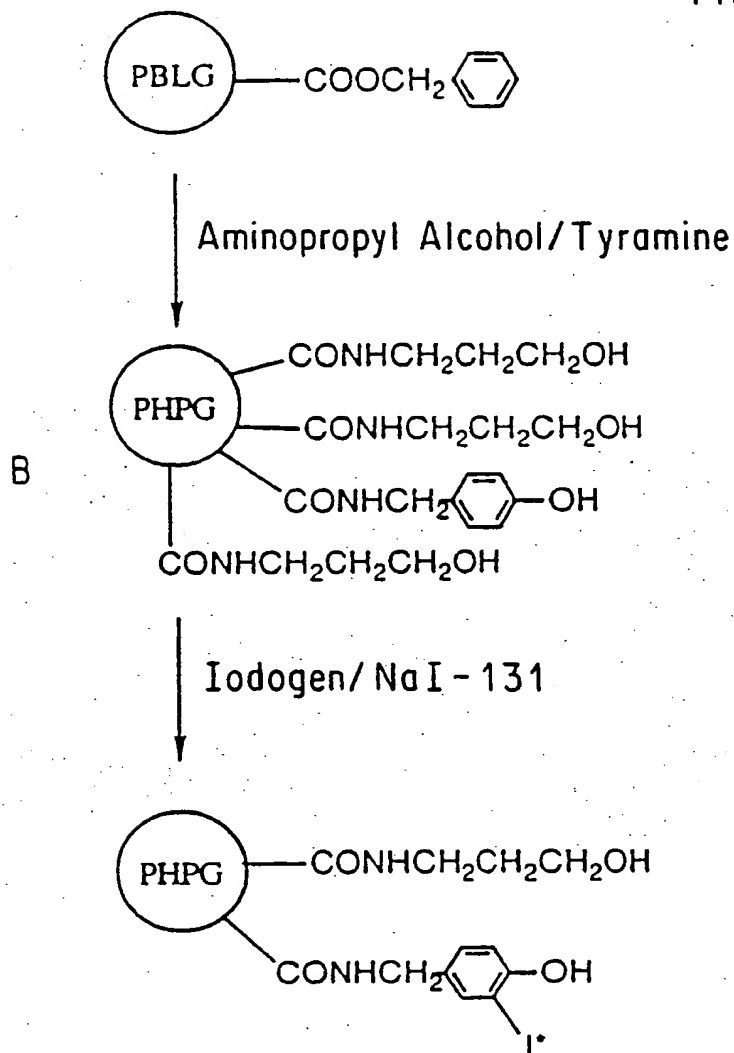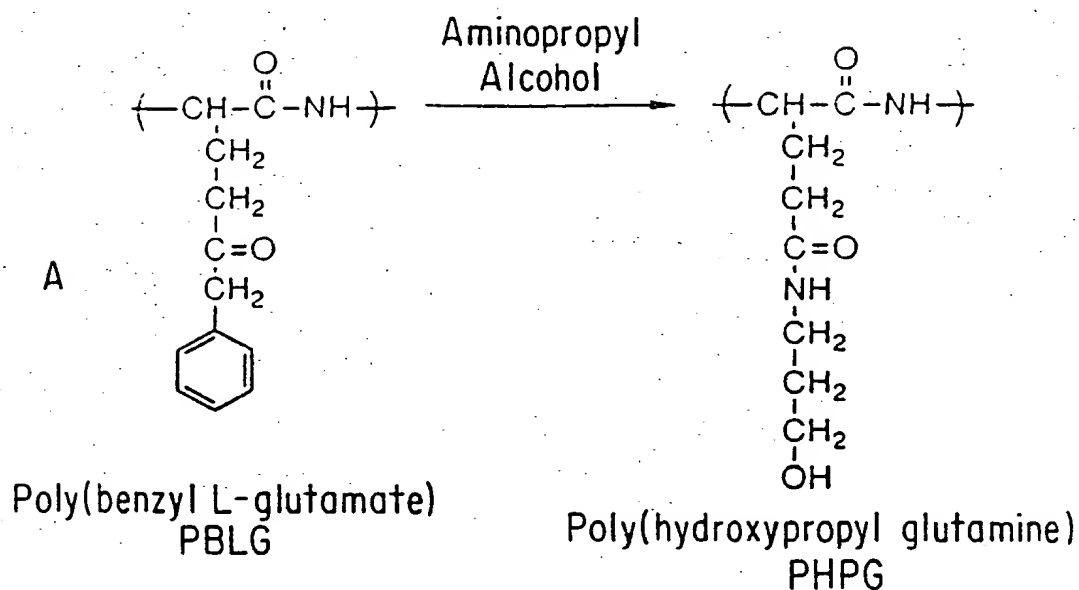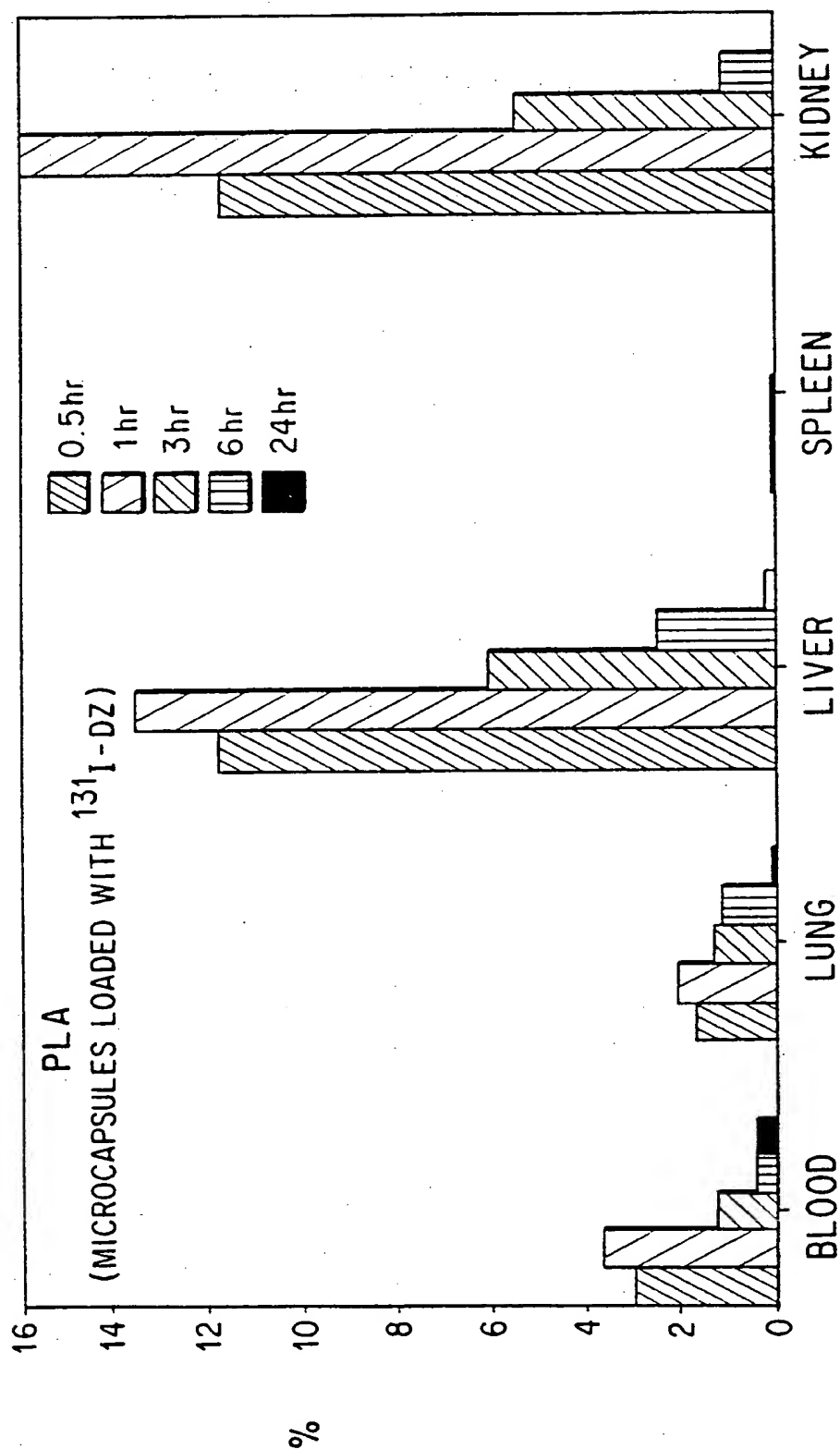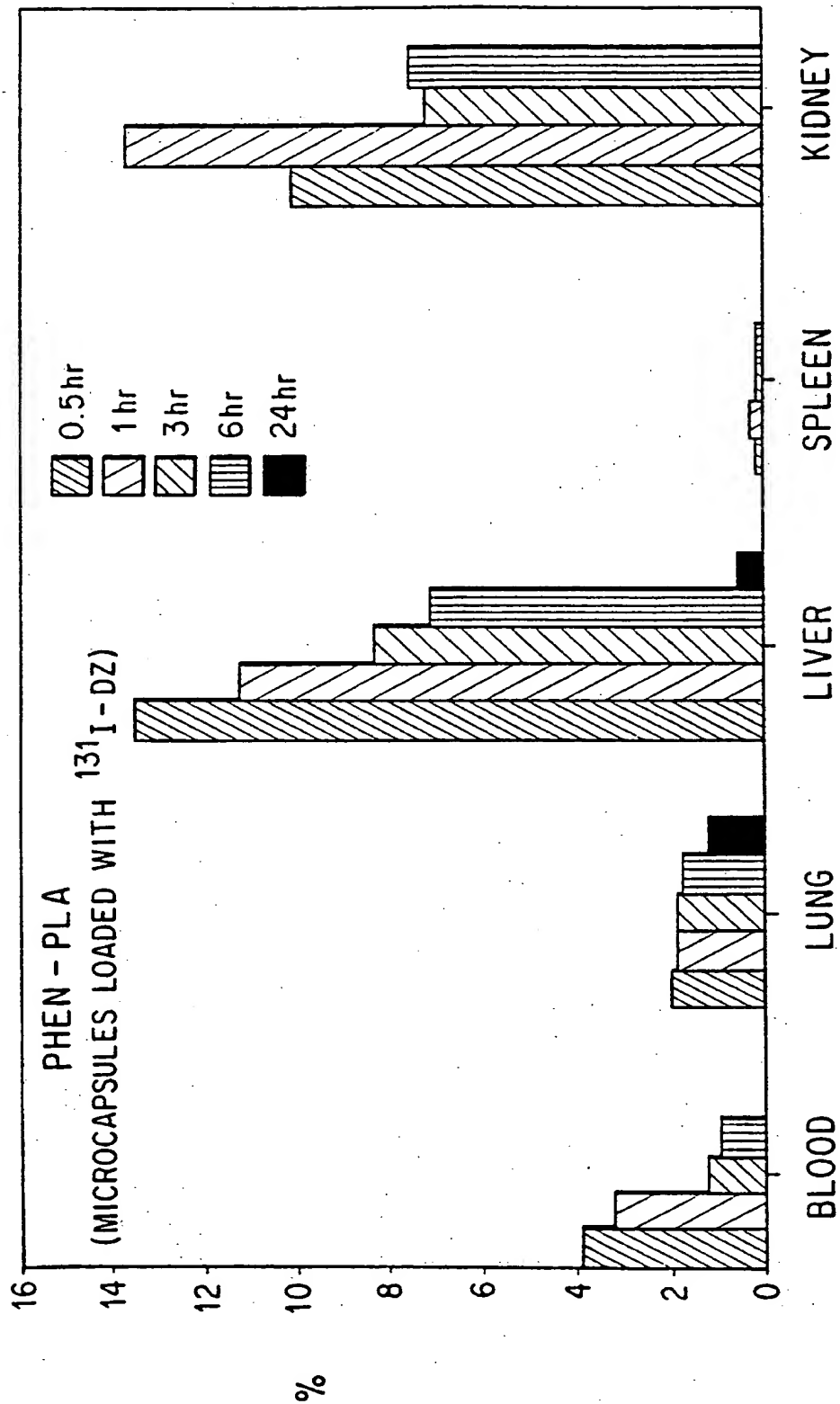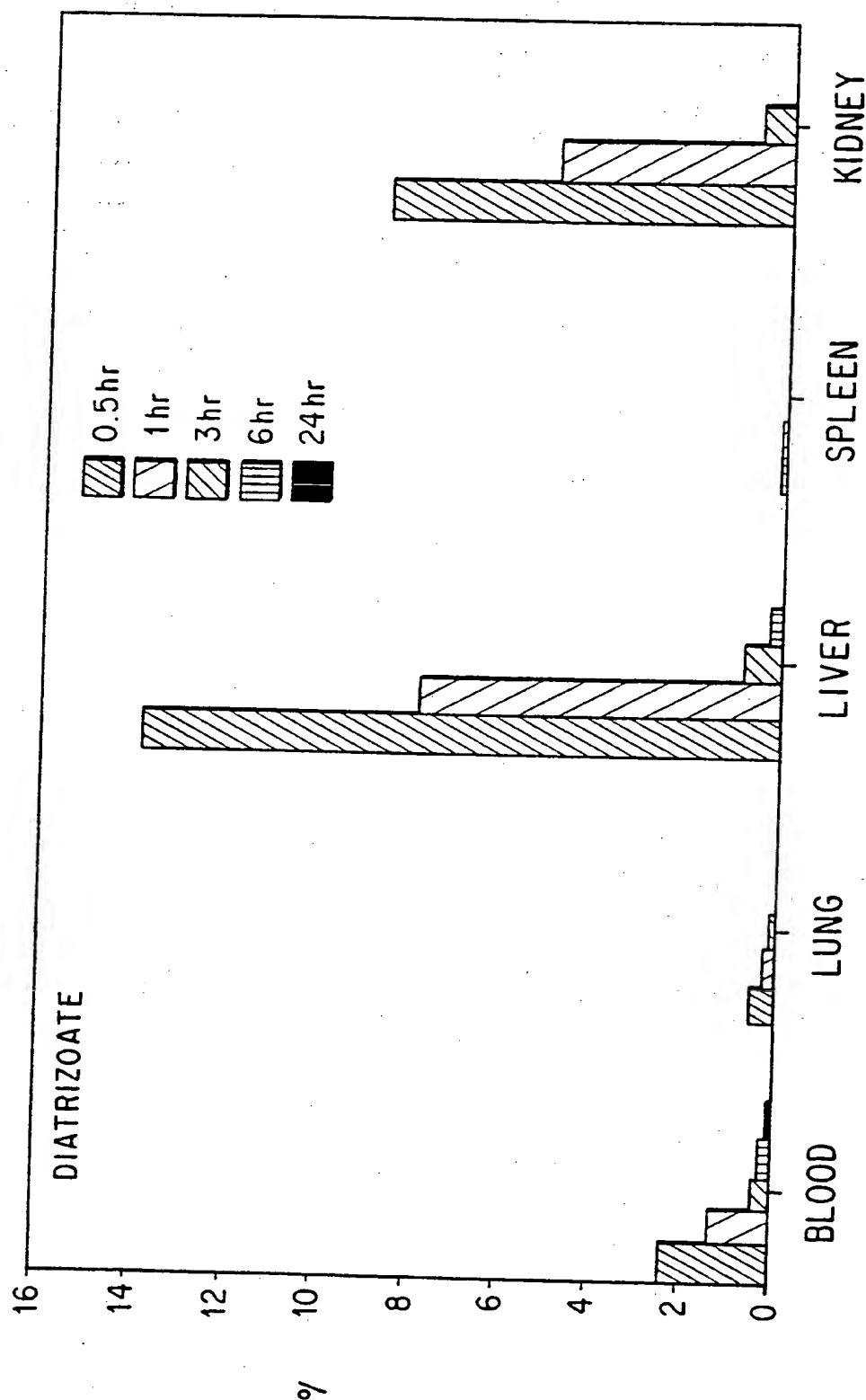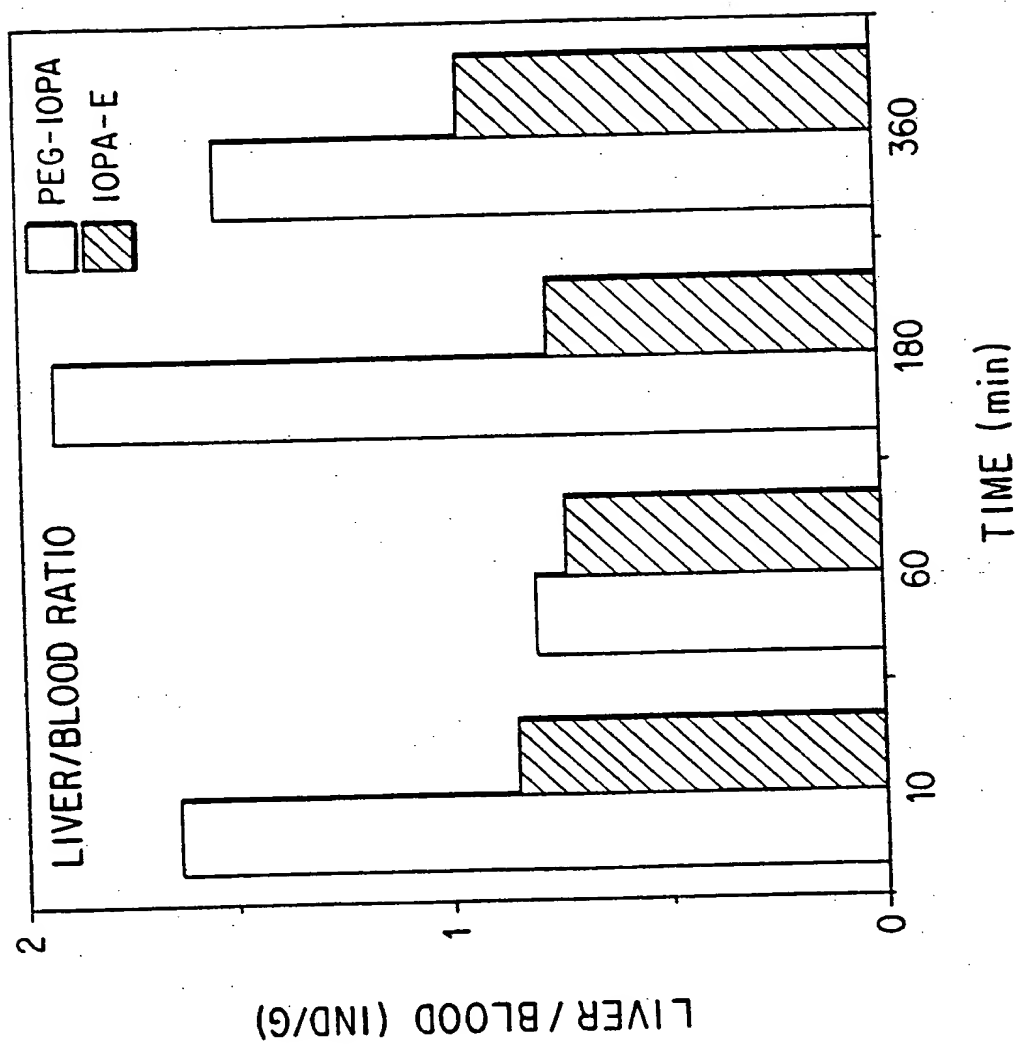
FIG. 21

F I G. 22A

FIG. 22B

FIG. 22C

FIG. 23

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US, A, 5,194,300 (CHEUNG) 16 MARCH 1993. SEE THE DRAWING SHEET; AND COLUMN 3, LINES 20-64.	
Y,E	US, A, 5,238,714 (WALLACE ET AL) 24 AUGUST 1993. SEE COLUMN 2, LINES 42-45 AND COLUMN 2, LINE 6 4 BRIDGING COLUMN 3, LINE 8.	6-8,11-14
A	US, A, 4,552,812 (MARGEL ET AL) 12 NOVEMBER 1985. SEE FIGURE 2.	
	US, A, 4,413,070 (REMBAUM) 01 NOVEMBER 1983. SEE FIGURE 2; AND COLUMN 3, LINE 33 BRIDGING COLUMN 4, LINE 16.	
A	US, A, 4,267,234 (REMBAUM) 12 MAY 1981.	
A	US, A, 5,069,936 (YEN) 03 DECEMBER 1991. SEE COLUMN 5, LINE 29 BRIDGING COLUMN 6, LINE 4; AND COLUMN 14, LINES 22-39.	
A	US, A, 4,138,383 (REMBAUM ET AL) 06 FEBRUARY 1979. SEE FIGURE 2; COLUMN 2, LINES 12-22; AND COLUMN 3, LINES 31-50.	

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A 61K 9/50, 49/00, 49/02; B01J 13/02
US CL :424/497.501,491,499,1.1,9
According to International Patent Classification (IPC) or to both national classificati n and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 428/402.21, 402.24; 264/4.3, 4.33; 427/213.31; 424/497,501,491,499,1.1,9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
X/Y	US, A, 4,671,954 (GOLDBERG ET AL.) 09 JUNE 1987: SEE COLUMN 2, LINES 20-46; COLUMN 3, LINES 14-26 AND LINES 36-54; COLUMN 4, LINES 51-68; AND COLUMN 5, LINES 38-44.	1-3,17-22/ 6-16,23-26
X/Y	US, A, 4,871,716 (LONGO ET AL) 03 OCTOBER 1989. SEE COLUMN 2, LINES 3-8, LINES 29-57; COLUMN 3, LINES 20-60; COLUMN 5, LINES 8-41 ABSTRACT.	1-3,17-22/ 6-16,23-26
A	US, A, 5,059,542 (HIRAI ET AL) 22 OCTOBER 1991. SEE COLUMN 2, LINES 18-27; COLUMN 3, LINE 59 THROUGH COLUMN 4, LINE 68; COLUMN 7, LINES 35-68; AND COLUMN 9, LINES 20-63.	
A	US, A, 4,230,685 (SENYEI ET AL) 28 OCTOBER 1980. SEE COLUMN 3, LINES 26-46.	

[X] Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 NOVEMBER 1993	JAN 0 7 1994

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